(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)


(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/082242 A2

(51) International Patent Classification: Not classified

(21) International Application Number: PCT/NZ2008/000340

(22) International Filing Date: 19 December 2008 (19.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/015,783 21 December 2007 (21.12.2007) US



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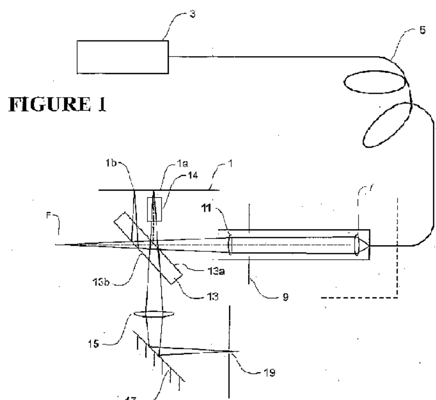
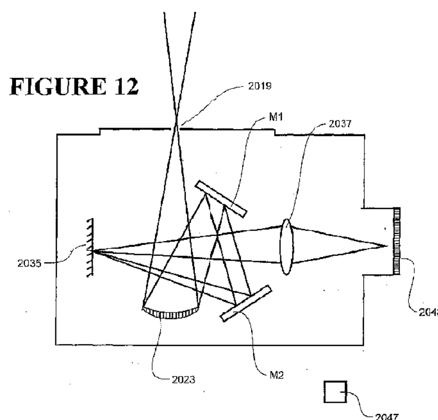
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *without international search report and to be republished upon receipt of that report*


(54) Title: ANALYSIS SYSTEM AND METHOD





**(57) Abstract:** An analysis system has a sample region (1), a source of excitation radiation (3), and a wavelength-inspecific beam splitter (13) to deliver some of the excitation radiation to a sample. A dispersion element (2023) disperses returned radiation from the sample to be analysed that has been transmitted by the beam splitter. A selectively switchable micromirror device (2035) receives returned radiation that has been dispersed by the dispersion element. A detector (2043) detects radiation that is received from the switchable micromirror device. The switchable micromirror device removes at least a major part of residual excitation radiation from the returned radiation that is passed to the detector. A method of quantifying target analyte(s) in a sample includes receiving radiation from a sample region for each of a plurality of points along at least part of the length of the sample region, and delivering at least some of the radiation to the detector.



WO 2009/082242 A2

# ANALYSIS SYSTEM AND METHOD

## FIELD OF INVENTION

The present invention relates to an analysis system and method. The analysis system and method are preferably suitable for quantifying at least one analyte in a sample.

## BACKGROUND

Samples are regularly analysed to determine the content of the samples. Conventional analysis systems are generally useful for analysing only one or a small number of targets in a single run.

Generally, the systems for quantifying multiple analytes require complex and/or expensive filtering arrangements to detect different targets. For example, many such systems rely on dichroic beam splitters to filter light of differing wavelengths to detect each target. The dichroic beam splitters are expensive. Additionally, they operate over only a narrow wavelength range; dichroic beam splitters generally change from reflective to transmissive over a wavelength range of about 20 nm. Accordingly, if dichroic beam splitters are to be used to detect multiple wavelengths, multiple beam splitters are required. This results in technically complex and expensive systems. Long pass filters to block leakage of incident radiation (laser light) may also be required.

It would be desirable to provide a system for analysing a sample that can accurately analyse the sample and that does not require extensive use of expensive dichroic beam splitters and long pass filters.

There are additional difficulties when there is a small amount of sample or the concentration of the analyte is low, particularly when quantification of a particular analyte is desired. Conventional technology used to achieve this goal includes microarrays, affinity chromatography and flow cytometry.

Standard microarrays and affinity chromatography systems require significant amounts of sample. Where sufficient level of sample or number of analytes is not available from the original sample, this problem is typically overcome by biological amplification of the analyte(s) in question such as PCR and RNA amplification. Amplification may distort the representation of different species in complex mixtures. Additionally, amplification is only available for analytes that can be amplified in a

straightforward manner, such as mRNA, cDNA and genomic DNA. Other biological analytes of interest, such as proteins, peptides, carbohydrates and lipids or non-biological analytes cannot be readily amplified.

Flow cytometry also has its disadvantages. While this technique may be used on smaller levels of analytes than standard microarrays and affinity chromatography systems, the labelled molecules in a flow cytometry system have relatively short "dwell time" in the detection "cell" where the fluorescent entity is exposed to the excitation radiation. This means there is a much shorter time in which all spectroscopic information on each analyte may be obtained and the system therefore needs a stronger signal.

It would be desirable to provide a system for quantifying one or more analyte(s) in a sample that does not require biological amplification of the analyte, and that can analyse analytes in parallel in adequate detail.

It is an object of at least preferred embodiments of the present invention to provide an analysis system or method that are suitable for use in analysing a sample, quantifying an analyte in a sample, or that at least provides the public with a useful choice.

## SUMMARY OF INVENTION

In a first aspect, the present invention broadly consists in an analysis system comprising:

a sample region for receipt of a sample to be analysed;

at least one source of excitation radiation; and

a wavelength-inspecific beam splitter that operatively couples the source of excitation radiation with the sample region to deliver some of the excitation radiation from the source of excitation radiation to a sample to be analysed in the sample region, the beam splitter configured to receive returned radiation from a sample to be analysed in the sample region and to transmit at least some of the returned radiation, wherein the returned radiation comprises residual excitation radiation and radiation to be analysed;

a dispersion element configured to disperse the returned radiation from the sample to be analysed that has been transmitted by the beam splitter;

a selectively switchable micromirror device configured to receive returned radiation that has been dispersed by the dispersion element; and

a detector to detect radiation that is received from the switchable micromirror device;

wherein the switchable micromirror device is configured to remove at least a major part of the residual excitation radiation from the returned radiation that is passed to the detector.

The system may comprise a single source of excitation radiation. The single source of excitation radiation may provide excitation radiation of only a single wavelength. Alternatively, the single source of excitation radiation may provide excitation radiation over a range of wavelengths. Alternatively, the system may comprise a plurality of sources of excitation radiation. Preferably, the source(s) of excitation radiation is/are a laser source. Preferably, the sources of excitation radiation comprise a plurality of laser sources, each of which is configured to provide an excitation radiation with a different wavelength or a different wavelength band. Any other suitable source(s) of excitation radiation could be used; for example mercury burner(s), xenon discharge lamp(s), or narrow band LED(s).

The beam splitter is preferably configured to reflect excitation radiation to the sample region (and to a sample therein). Preferably, the beam splitter is configured to reflect only a small proportion of the excitation radiation to the sample region (and to a sample therein), and allows the majority remainder of the excitation radiation to pass through the beam splitter. Preferably, a surface of the beam splitter is configured to reflect between about 5% and about 15% of the excitation radiation to the sample region (and to a sample therein). Most preferably, the surface of the beam splitter is configured to reflect about 10% of the incoming excitation radiation to the sample region (and to a sample therein). Preferably, at least a major part of the returned radiation from the sample to be analysed that is delivered to the beam splitter, passes through the surface of the beam splitter. Preferably, about 85 to about 95% of the returned radiation that is delivered to the beam splitter passes through the surface of the beam splitter. Most preferably, about 90% of the returned radiation that is delivered to the beam splitter, passes through the surface of the beam splitter.

The beam splitter may be made of any suitable material. In a preferred embodiment, the beam splitter is made of a glass material, such as BK7 glass for example, which offers substantially linear optical transmission from about 2000 nm down to about 350 nm. The beam splitter could be made of any other suitable material, such as a different type of glass, or a plastic material for example.

Preferably, the beam splitter is made of a material which offers substantially linear optical transmission over a range of at least about 350 nm to about 800 nm, more preferably over a range of at least about 350 nm to about 1000 nm, most preferably over at a range of at least about 350 nm to about 1400 nm.

The surface of the beam splitter that reflects the excitation radiation to the sample region may be a front surface of the beam splitter, and the beam splitter may also have a rear surface that reflects and transmits in similar proportions to the front surface.

The beam splitter will preferably be configured so that excitation radiation being reflected by the rear surface of the beam splitter toward the sample region does not significantly interfere with the excitation radiation being reflected by the front surface of the beam splitter toward the sample region. As such, the beam splitter will preferably be configured to have a sufficient thickness, such as at least about 2 mm for example, to prevent or minimise interference. In a preferred form, the beam splitter has a thickness of about 10 mm. Alternatively, the beam splitter may be sufficiently thin that the excitation radiation being reflected is directed to the sample in the sample region. In that configuration, the beam splitter preferably has a thickness of between about 60 and about 150 micrometers, more preferably between about 80 and about 100 micrometers.

The dispersion element may be any suitable form that spatially disperses the returned radiation from the sample to be analysed. For example, the dispersion element could be a prism or a grating. In a preferred form, the dispersion element is a diffraction grating that spatially disperses the returned radiation depending on the wavelengths or wavelength ranges within the returned radiation. Preferably, the dispersion element is a concave holographic grating that receives the returned radiation and disperses the returned radiation onto the SMD.

The switchable micromirror device (SMD) is preferably a digital micromirror device (DMD) or a digital light processing (DLP) device. Preferably, the SMD has a plurality of columns of micromirrors and a plurality of rows of micromirrors. The SMD is preferably arranged so that either each column or each row of micromirrors, or a plurality of adjacent columns or rows of micromirrors, corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element. Most preferably, the SMD is arranged so that each column or a plurality of adjacent columns, of micromirrors corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element.

The SMD may be controlled by any suitable means, such as via a processing unit that is programmed to control the SMD. The dispersed returned radiation from the sample to be analysed is focussed onto the SMD, and the SMD will be controlled to remove at least a major part of the residual excitation radiation from the returned radiation, so that little or no residual excitation radiation is passed to the detector. When the SMD is arranged so that each column, or plurality of adjacent columns, of micromirrors corresponds to a respective wavelength or wavelength range, at least a major part of the residual excitation radiation can be removed from the radiation passed to the detector by turning one or more columns of the SMD corresponding to the residual excitation radiation line. The SMD may be controlled so that the radiation to be analysed is passed to the detector a single column, or a plurality of adjacent columns, at a time, so that radiation corresponding to a single wavelength or wavelength range is passed to the detector at a time. However, the SMD may be controlled to remove only the residual excitation radiation, and to direct all of the radiation to be analysed to the detector at once. While that configuration has broad applications, it is particularly useful if the system is used with a sample that photodegrades.

The system could comprise a plurality of SMDs if required in series and/or in parallel.

As returned radiation having different wavelengths will be deflected a differing amount by the dispersion element, the dispersion element may be able to be actively turned to direct a different wavelength toward the SMD. Alternatively, the system may comprise a plurality of dispersion elements at different angles, to direct the returned wavelengths of the various angles toward the SMD. These alternatives will only be required if the returned radiation to be analysed covers a sufficiently wide wavelength range that it would not otherwise all be directed onto the SMD.

Preferably, the system comprises plurality of dispersion elements in a stack, with each dispersion element oriented at a different angle relative to the other dispersion elements.

The system may comprise one or more suitable optical components between any of the other components mentioned above. For example, the system may have one or more reflective and/or one or more refractive elements between any of the other components mentioned above. Additionally, one or more optical fibres may be used between components to enable the components to be spaced remotely from one another.

The configuration of the system will be such that the dispersed returned radiation to be analysed is focussed onto the SMD, preferably such that the wavelength(s) of returned radiation to be analysed correspond to respective columns or rows of micromirrors of the SMD.

In a preferred embodiment, the source of excitation radiation may be operatively coupled to the beam splitter by a collimating lens. An optical fibre may direct excitation radiation from the source of excitation radiation to the collimating lens, and the collimating lens will cause the diverging excitation radiation that is exiting the optical fibre to become substantially parallel. A focusing lens may be provided and will cause the substantially parallel excitation radiation to converge. Generally, the focus of the focusing lens will be beyond the beam splitter. Preferably, the focusing lens and a microscope section objective lens are configured to focus the excitation radiation on the sample region, and the beam splitter turns some of the excitation radiation from the focusing lens to pass through the microscope section objective lens.

If multiple sources of excitation radiation are provided, the excitation radiation from each of the sources will preferably be delivered to the sample region by the wavelength-inspecific beam splitter. The excitation radiation from the multiple sources could be delivered to the beam splitter by any suitable mechanism. For example, where the sources of excitation radiation are a plurality of lasers in a laser bank, dichroic lenses could be provided in the laser bank to deliver the excitation radiation from the multiple sources into the system, via a single optical fibre for example. As an alternative example, each source of excitation radiation could be operatively coupled to a respective optical fibre, with the plurality of optical fibres operatively coupled into a single optical fibre to feed the excitation radiation from the multiple sources to the beam splitter. Any other suitable configuration could be used.

The source(s) of excitation radiation will be configured to supply radiation at desired wavelength(s) corresponding to fluorescence(s) of the analyte(s) to be detected. By way of example only, the excitation radiation wavelength(s) may be within the range of about 400 nm to about 800 nm, possibly within the range of about 400 nm to about 1000 nm, possibly within the range of about 400 nm to about 1400 nm. For example, the excitation radiation(s) may be one or more of 405 nm, 473 nm, 475 nm, 532 nm, 593 nm, 633 nm, 635 nm, 650 nm, or 780 nm.

The returned radiation that passes through the beam splitter will generally be diverging, and a second focusing lens will preferably be provided to cause the returned radiation to focus. In a preferred form, the beam splitter and sample region are part of a microscope section of the system; and the dispersion

element, SMD, and detector are part of a spectrometer section of the system. Preferably, the second focusing lens is configured to focus the returned radiation into an entrance of the spectrometer section. The entrance may be in the form of a pin hole or entrance slit. A mirror may be provided to direct the returned radiation from the second focusing lens to the entrance slit. The entrance slit of the spectrometer section is preferably confocal with the sample region of the microscope section.

The microscope section will preferably comprise an objective lens between the beam splitter and the sample region. The objective lens may be separated from the sample region by an air gap. Alternatively, the objective lens could be coupled to the sample region by oil, to reduce the number of reflective surfaces. The objective lens may be a magnifying lens, and could be provided with any suitable magnification, such 10x, 60x, or any other suitable magnification. The objective lens may be adjustable or replaceable to adjust the magnification.

In an alternative embodiment, the apparatus may not comprise a microscope section, and instead the beam splitter, focusing lens, and objective may be incorporated into the spectrometer section.

In one embodiment, the returned radiation will be diverging from the entrance slit, and is preferably received by a collimating mirror to make the returned radiation substantially parallel. The substantially parallel returned radiation is directed by the collimating mirror to the dispersion element. In an alternative embodiment, the returned radiation passing through the entrance slit may propagate through free space to a concave holographic grating.

The spatially dispersed returned radiation from the dispersion element is preferably directed to one or more mirrors, and a third focusing lens may be provided to focus that returned radiation onto the SMD. One or more mirrors may be provided between the third focusing lens and the SMD to direct the returned radiation.

The radiation to be analysed from the SMD may be directed to one or more mirrors which deflect the radiation to the detector. A focusing lens is preferably provided to focus the radiation to be analysed onto the detector.

The detector and/or any intervening optical component(s) may be configured relative to the SMD so that when a micromirror of the SMD is not tilted, returned radiation will be passed from that micromirror to the detector. Alternatively the detector and/or any intervening optical component(s)

may be configured relative to the SMD so that when a micromirror of the SMD is tilted, returned radiation will be passed from that micromirror to the detector.

A concave holographic grating could be provided and configured to receive the radiation to be analysed from the SMD and focus that onto the detector. That would operate in reverse to the holographic mirror that disperses that onto the SMD, such that the dispersed spectrum of radiation to be analysed from the SMD is focussed onto a point detector.

The detector may be any suitable type, such as a charge-coupled device (CCD) for example. Other suitable types of detector could be used. For example, an indium gallium arsenide (InGaAs) detector, silicon detector, or a small photodetector (such as an avalanche photodiode) or point detector could be used. The small photodetector or point detector may be suitable in a system having a concave holographic grating that focuses the returned radiation to be analysed from the SMD onto the detector.

The system is preferably configured to determine from the radiation to be analysed that is received by the detector, analysis data relating to the received radiation. The system is preferably configured to determine from the radiation received by the detector, the quantity of one or more analytes in a sample. The system is preferably configured to quantify at least five analytes in a sample from a single source of excitation radiation. The system is preferably configured to quantify twenty analytes in a sample, from four sources of excitation radiation.

The radiation to be analysed that is received by the detector will suitably be deconvoluted or decomposed to provide analysis data relating to the received radiation. For example, the radiation received by the detector may be deconvoluted to quantify one or more analytes in a sample, where the analytes are labelled directly or indirectly with fluorescence. The system will be provided with a processing unit that is programmed to determine the quantity of one or more analytes, by providing the desired deconvolution or decomposition, for example.

In a preferred embodiment, the processing unit will be programmed to enable the system to provide a quantification measurement of at least one, preferably at least two, preferably at least three, preferably at least four, and more preferably at least five, analytes in a sample from a single excitation radiation. For example, the system is preferably configured to provide a quantification measurement of up to five analytes in a sample from a single laser line. When multiple sources of excitation radiation are provided, the system is preferably configured to provide a quantification measurement of up to five

analytes in a sample from each source of excitation radiation. Quantification of five analytes from each source of excitation radiation is believed to be optimal; however, a greater or lesser number of analytes could be quantified from each source of excitation radiation. As such, if four laser lines are provided, the system is preferably configured to provide a quantification measurement of up to twenty analytes in a sample, and so on. More than four laser lines could be used. When the bandwidth of excitory peaks is sufficiently large, some fluorophores may be caused to fluoresce by more than one of the sources of excitation radiation.

The system is preferably configured to provide a quantification measurement of an analyte in the sample, based on total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, for a plurality of points in the sample region.

The system may have more than one detector. For example, the system may have two detectors, each configured to receive radiation from respective portions of the SMD so that a plurality of wavelengths or wavelength bands can be analysed concurrently, one per detector. That could enable the system to operate more rapidly and compactly than if a single detector is used.

The system is preferably configured to provide relative movement between the incoming excitation radiation and the sample region, so that the excitation radiation can be directed to selected areas of the sample region or scanned along the sample region. Preferably, the sample region is selectively moveable relative to the beam splitter, to enable the system to scan the excitation radiation over a desired area of the sample. The sample region will suitably comprise a motorised stage that may be controlled by a suitable controller to enable the system to scan excitation region over a desired area of the sample.

The system could be used for any other suitable analysis. By way of example, the system could be used to detect analyte(s) in a sample. Preferably the system is used to quantify analyte(s) in a sample. The system is preferably used to quantify analyte(s) in the sample by the method of the third aspect below.

Alternatively, the system could be used for any suitable analysis. By way of example only, the system could be used for Raman spectroscopy, to analyse the purity of a pharmaceutical composition for example. By providing a pinhole in the system, the system could be used as a confocal microscope. The sample region will be configured according to the purpose the system will be used for and the type of sample to be analysed.

The analysis system may be configured to provide a quantification measurement of an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, corresponding to the analyte, to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

Alternatively, the analysis system may be configured to provide a quantification measurement of an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, corresponding to the analyte to programmed factor(s) relating to the analyte.

In a second aspect, the present invention broadly consists in a method of analysing a sample, comprising:

providing an analysis system of the first aspect above;

positioning the sample in the sample region;

delivering excitation radiation to the sample via the wavelength-inspecific beam splitter to generate returned radiation comprising residual excitation radiation and radiation to be analysed;

receiving dispersed returned radiation from the dispersion element on the switchable micromirror device; and

operating the switchable micromirror device to remove at least a major part of the residual excitation radiation from the returned radiation that is passed to the detector but to deliver at least some of the radiation to be analysed to the detector.

With respect to the first and second aspects of the present invention, the sample region may comprise any suitable sample region, such as a microarray, an optically-transparent surface (e.g. cover-slip or glass slide), or the sample region of the third aspect for example.

The sample may contain one or more target analyte(s). The target analyte(s) may be fluorescently-labelled, where the fluorescent labels receive the excitation radiation and return at least some of that radiation to the beam splitter. Where no target analyte is present, there will be no returned radiation at the particular wavelength or wavelength range corresponding to the fluorescence, and therefore a "negative" result.

Preferably, the sample contains one or more target analyte(s), and the method comprises quantifying the target analyte(s) in the sample. Preferably, the sample contains five target analytes, and the method comprises quantifying the five target analytes. Preferably, the sample contains twenty target analytes, and the method comprises quantifying the twenty target analytes.

The target analyte(s) in the sample may be labelled in any suitable way. Preferably the target analytes are labelled as described with reference to the third aspect.

The method preferably comprises quantifying at least one target analyte in accordance with the third aspect below. In alternative embodiments the method may be used for any suitable means, including use in Raman spectroscopy.

The method may comprise quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to the analyte, to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

The method may alternatively comprise quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to programmed factor(s) relating to the analyte.

In a third aspect, the present invention broadly consists in a method of quantifying at least one target analyte in a sample, comprising:

providing a sample region for receipt of a sample to be analysed, the sample region comprising a group of receptors along a length of the sample region that are directly or indirectly immobilised relative to a surface of the sample region;

providing an analysis system comprising a detector;

providing a sample to be analysed;

passing the sample along the length of the sample region comprising receptors, whereby a specific analyte in the sample, if any, binds to the receptors;

labelling the target analyte either before or after passing the sample along the length of the sample region comprising receptors;

receiving radiation from the sample region for each of a plurality of points

along at least part of the length of the sample region or different parts of the sample region and delivering at least some of the radiation to the detector; and

quantifying the target analyte in the sample, if any, from the radiation passed to the detector.

Preferably, the group of receptors comprises a plurality of types of receptor, each type of receptor being specific for a different target analyte, and at least two analytes are quantified. Preferably, the group of receptors comprises at least three types of receptors specific for at least three target analytes and at least three target analytes are quantified. Preferably, the group of receptors comprises at least four types of receptors for at least four target analytes and at least four target analytes are quantified. More preferably, the group of receptors comprises at least five types of receptors specific for at least five target analytes and at least five target analytes are quantified. Most preferably, the group of receptors comprises at least twenty types of receptors specific for at least twenty target analytes and at least twenty target analytes are quantified.

The plurality of types of receptors may be substantially homogenously mixed along the length of the sample region. Alternatively, the sample region may have separate distribution zones for each type of receptor along the length of the sample region.

The sample region is suitable for receipt of the sample while the method is performed. For example the sample region may comprise any suitable chamber, such as a tube, capillary, column or cuvette for example. The chamber may be open at one or both ends. A majority of the sample, other than the analyte that has bound to the receptors, may pass through and exit the sample region. In the preferred form, the sample region is a capillary tube having a rectangular cross-section with a height of about 50 micrometers, a width of about 500 micrometers, and a length of about 10 mm. Alternatively, the tube has a width of from about 1 micrometer to about 2 millimetres and a height of about 0.5 millimetres to at least about 50 millimetres. Preferably the column has a width of about 100 micrometres by 80 micrometres and a length of about 12 millimitres or 25 millimetres. The tube could have any other suitable dimensions or configuration.

The sample region may be reusable or disposable.

The sample region may comprise a matrix comprising bound receptors. The sample region may comprise a matrix of beads with bound receptors. The matrix maybe positioned in a receptacle for

example. Preferably the beads are magnetic and are held in place by a magnet. Alternatively, the matrix may be retained in place by a porous substrate such as cotton wool or nylon mesh.

The receptors may comprise any suitable chemical entity that will bind to the analyte such as probes, ligands or antibodies, for example. The receptors may be specific for a target analyte. For example, the receptors may be monoclonal antibodies or nucleic acid probes, preferably cDNA probes, that are produced or selected for their specificity to bind to the target analyte. The receptors may alternatively be streptavidin-coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex.

The receptors may be directly or indirectly immobilised on a surface on the sample region.

Where the receptors are directly immobilised on a surface of the sample region, the surface may be inherently adsorbent for the receptors or may require chemical activation or modification. Where chemical activation or modification is required, glass surfaces may be treated with a range of silanising reagents that introduce amino, carboxy, sulphydryl and other chemical groups that can be used to attach receptors.

Where the receptors are indirectly immobilised on a surface of a sample region, this may be achieved through any suitable method. In a preferred embodiment, beads which attach to the receptors may be attached to the surface of the sample region. The beads may be attached to a surface of the sample region before or after being attached to the receptors. The beads may be magnetic beads and attached to a surface of the sample region using magnetic attraction, for example using a powerful permanent magnet such as a neodymium magnet. Alternatively, the beads may be covalently attached to the surface, or indirectly immobilised by a matrix such as the type described above for example.

The beads are preferably beads or microspheres with a diameter of between about 1 and about 100 micrometers, preferably, about 40 micrometers. More preferably the beads have a diameter of between about 1 and about 30 micrometers, even more preferably the beads have a diameter of between about 1 and about 8 micrometers. Most preferably the beads are diamagnetic beads with a diameter of 1.05 micrometers or 5.91 micrometers. The beads within the sample region may range in size.

In another alternative the receptors are on the surface of beads or particles that are uniformly packed into the sample region.

The sample may be any biological or non-biological sample where knowing the quantity of at least one specific analyte is desired. When the sample is a biological sample, it may be human or non-human and it may be selected from any biological material including blood, saliva, semen, vaginal secretion, urine, faecal material, for example, or cells including embryonic cells for example, or organs or parts of organs for example, or mixtures thereof. Alternatively the sample may be taken from soil, water or air for example.

The sample may contain one or more target analytes to be quantified.

Preferably, the method of quantifying includes a pre-concentrating step, which concentrates target analytes within the sample before analysing such analytes.

Preferably, the pre-concentrating step uses an ion exchanger to bind analytes. A concentrate is obtained by releasing the bound analytes from the ion exchanger in a very small volume. For example, the bound analyte may be released by changing the pH conditions of the environment in which the analyte is bound. Alternatively, the bound analyte may be released by other methods such as increasing the salt concentration of the environment in which the analyte is bound.

Preferably, the ion exchangers include cleavable linkers joining an ionic group to a solid support. Preferably, the solid support is a bead. Preferably, the analyte is released from the ion exchanger by cleavage of the linker.

Preferably, the method comprises analysing a sample to quantify one analyte in the sample. More preferably, the method comprises analysing a sample to quantify each of several analytes in the sample. Preferably, the method comprises analysing a sample to quantify each of at least two, more preferably at least five, and most preferably at least twenty analytes in a sample.

Preferably the target analyte(s) is/are protein(s), peptide(s), DNA, RNA, carbohydrates(s), lipid(s), or mixtures thereof. Where the sample is a biological sample, the analyte(s) may be one or more foreign bodies such as a drug(s). More preferably, the target analyte is a protein, peptide or nucleic acid.

The target analyte(s) in the sample may be labelled in any suitable way where the analysis system can quantify the analyte(s) from information received from the labels.

For example, the target analyte(s) may be fluorescently labelled, labelled using chemically-driven light-emitting systems that are non-fluorescent such as luciferases for example, or labelled using non-electromagnetic radiation such as magnetism. The target analyte may be labelled indirectly or directly. Preferably, the target analyte is labelled with a labelled antibody. In alternative embodiments the target analyte(s) may be enzymes or enzyme conjugates that generate fluorescent products. For example the fluorescent products may be aptamers such as DNA or RNA molecules that quench or unquench when complementary DNA or RNA strands bind or the aptamer is folded or unfolded.

Most preferably the target analyte(s) are fluorescently labelled, where the fluorescent labels receive the excitation radiation and return at least some of that radiation to the detector. Where no target analyte is present, there will be no returned radiation at the particular wavelength or wavelength range corresponding to the fluorescence, and therefore a "negative" result.

Preferably the fluorescent label comprises a fluorophore-receptor complex. The receptor of the fluorophore-receptor complex may comprise any suitable chemical entity that will bind to the target analyte(s) such as probes, ligands or antibodies for example. The receptors may be specific for a target analyte. For example, the receptors may be monoclonal antibodies or nucleic acid probes that are produced or selected for their specificity to bind to the target analyte. The receptors may alternatively be streptavidin-coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex.

Where the target analyte(s) is/are labelled through a receptor such as a receptor-fluorophore complex, the immobilised receptors are primary receptors and the receptors of the fluorophore-receptor complexes are secondary receptors.

Primary receptors are generally bound to a surface whereas the secondary receptors are generally soluble and bind to the analyte bound to the primary receptor. The secondary receptor may be directly bound to a fluorophore. Alternatively, it may be indirectly bound. For example, a fluorophore-labelled tertiary receptor may bind to the secondary receptor to form a complex comprising a first receptor bound to the surface of the sample region, analyte, secondary receptor and labelled tertiary receptor.

This approach could for example use a first and a second antibody to each bind the analyte. That complex may then be subsequently labelled by addition of a third labelled antibody, the third antibody being specific to bind the second antibody. The third antibody can be, for example, a goat or rabbit fluorescent anti-mouse IgG. The use of different labelled antibodies specific for different secondary antibodies allows the quantification of different analytes in a sample and the different labels can be measured.

Where the method involves quantification of more than one target analyte, the method must allow for different target analytes to be identified. This may be achieved in a number of ways.

In a preferred embodiment, the method may allow for the quantification of different analytes where the labels are specific for target analytes. For example, if the analytes are labelled through receptor-fluorophore complexes, specific receptors may be attached to specific fluorophores. Where the quantity of two analytes is desired, a first group of receptors of a receptor type specific for the first analyte is attached to fluorophores that return radiation at a first wavelength or wavelength band and a second group of receptors of a receptor type specific for the second analyte are attached to a second group of fluorophores that return radiation at a second wavelength or wavelength band. Where the quantity of three analytes is desired, a first group of receptors of a receptor type specific for the first analyte is attached to fluorophores that return radiation at a first wavelength or wavelength band, a second group of receptors of a receptor type specific for the second analyte are attached to a second group of fluorophores that return radiation at a second wavelength or wavelength band, and a third group of receptors of a receptor type specific for the third analyte are attached to a third group of fluorophores that return radiation at a third wavelength or wavelength band, and so on. In this embodiment the analysis system can distinguish between different analytes by deconvoluting the returned radiation with reference to different wavelengths or wavelength bands each corresponding to a particular fluorescence that is associated with a particular analyte in the sample.

In an alternative embodiment, the immobilised receptors are specific for a target analyte and the analysis system can detect the difference between different receptor types. For example, if the receptors are indirectly immobilised on a surface of the sample region through beads, and the quantity of two analytes is desired, then a group of receptors of a receptor type specific to the first analyte may be attached to a group of beads of a first colour, and a group of receptors of a receptor type specific to the second analyte may be attached to a group of beads of a second colour. Where the quantity of three analytes is desired, the third group of receptors of a receptor type specific for the third analyte

may be attached to a group of beads of a third colour, and so on. In this embodiment the analysis system can distinguish between different analytes by deconvoluting or decomposing the spectrum of the returned radiation with reference to the different groups of coloured beads.

Where reference is made herein to groups of beads of different colours, each bead of one group of beads may have the same colour, which is a spectrally different colour to each bead of another group of beads. Alternatively, each bead of one group may have a different combination of colours to each bead of another group.

In one embodiment, a combination of both immobilised receptors attached to coloured beads and fluorophores of different wavelengths may be used to quantify a plurality of analytes in a sample. The immobilised receptors and receptor-fluorophore complexes together have a certain level of specificity that allows for a large number of different analytes to be quantified in the same sample.

The target analyte(s) may be labelled either before or after passing the sample along the length of the sample region comprising receptors.

Where the target analyte(s) is/are labelled before passing the sample along the length of the sample region comprising receptors, labels may be added to the sample and the sample allowed to incubate for a period sufficient to ensure binding. When the target analyte(s) is/are labelled after the sample has been passed along the length of the sample region, the labels may be passed along the length of the sample region with a flow rate that allows for the labels to bind to the target analyte.

The analysis system may be any suitable system that can provide an intensity for a particular wavelength that can be deconvoluted to quantify target analytes in a sample. Preferably the analysis system is the analysis system of the first aspect as outlined above.

The analysis system may be any suitable system comprising a detector that can be used to quantify the target analyte in the sample. When the analyte(s) is/are labelled fluorescently, the analysis system preferably comprises at least one source of excitation radiation. Preferably, the excitation radiation is directed into the sample region in a direction transverse to the length of the sample region comprising receptors, and is moved along at least part of the length of the sample region comprising receptors. The excitation radiation may additionally be moved across at least part of the width of the sample region comprising receptors. The detector receives at least some of the returned radiation.

In a preferred embodiment, the amount of target analyte bound to the group of receptors generally reduces along at least part of the length of the sample region comprising receptors so that more target analyte is bound to the sample region where the sample was administered than at the end opposite to where the sample was administered due to the previous binding of the analyte to the receptors. Accordingly, the intensity of returned radiation relating to the fluorescence of the analyte that is delivered to the detector will generally reduce along the length of the sample region from the first end as the number of unbound analyte(s) in the sample reduces due to binding with the immobilised receptors. Beyond a certain point, there may be no binding, and the excitation radiation will not generate any returned radiation. This creates an intensity profile that changes along the length of the sample region, and this information can be used to quantify the level of analyte in the sample. Where the quantity of more than one analyte is being analysed, different analytes may travel to different points along the sample region through progressive exhaustion of binding sites by the analyte.

The method preferably comprises quantifying an analyte in the sample, based on total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, for a plurality of points along the sample region. Preferably the method comprises quantifying a plurality of analytes in the sample, based on total intensity of the radiation to be analysed received by the detector at a plurality of wavelengths or wavelength ranges, for a plurality of points along the sample region.

The method may further comprise determining the concentration of one or more analyte(s) in a sample by quantifying the analyte as described above, and subsequently determining the concentration with reference to the volume of sample passed along a length of the sample region.
The method may comprise quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

Alternatively, the method may comprise quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to programmed factor(s) relating to the analyte.

In a fourth aspect, the present invention broadly consists in an apparatus configured to perform the method of the third aspect.

In a fifth aspect, the invention broadly consists in an apparatus configured to quantify two or more target analytes in a sample. Preferably the target analytes are protein(s), peptide(s), DNA, RNA, carbohydrate(s), lipid(s), or mixtures thereof. Preferably the apparatus is the apparatus of the first aspect and is configured to carry out the method of the third aspect.

The invention consists in the foregoing and also envisages constructions of which the following gives examples only.

## BRIEF DESCRIPTION OF DRAWINGS

Preferred embodiments of the present invention will now be described by way of example only and with reference to the accompanying drawings in which:

Figure 1 shows a schematic view of a microscope section of a preferred embodiment analysis system;

Figure 2 shows a schematic view of a spectrometer section of the preferred embodiment analysis system;

Figure 3 shows a schematic view of a preferred embodiment method for quantifying analyte in a sample;

Figure 4 shows a schematic view of a sample region being used in a preferred embodiment method of quantifying analyte in a sample;

Figure 5 shows a schematic view of the sample region when used to quantify analyte in the sample;

Figure 6a shows an example plot of intensity versus wavelength at point 101a of the sample region;

Figure 6b shows an example plot of intensity versus wavelength at point 101b of the sample region;

Figure 7a shows an example plot of intensity versus point along the sample region for a target analyte, with this data having been extracted from the data of Figures 6a and 6b;

Figure 7b shows an example plot of intensity versus point along and across the sample region for a wavelength or wavelength band corresponding to a target analyte;

Figure 8a shows a schematic plan view of part of an alternative preferred form system that includes a plurality of dispersion elements;

Figure 8b shows an example of the excitation radiation that may be received by the SMD with the dispersion element configuration of Figure 8a;

Figure 9 shows a plot of radiation intensity for Experiment 1;

Figure 10 shows results from Experiment 2 at one position along the sample region;

Figure 11 shows relative signal strengths for different beads along the sample region;

Figure 12 shows a schematic view of an alternative spectrometer section for an alternative preferred embodiment spectrometer section;

Figures 13a to 13f show intensity plots for a 0.25μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 14a to 14e show intensity plots for a 0.5μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 15a to 15g show intensity plots for a 0.75μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 16a to 16h show intensity plots for a 1μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 17a to 17k show intensity plots for a 2μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 18a to 18p show intensity plots for a 3μL aliquot of a 150 μM stock of fluorescein made up to 10μL. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figures 19a to 19p show intensity plots for a 10μL aliquot of a 150 μM stock of fluorescein. Each plot shows the intensity at a different interval 0.5mm along the length of a tube prepared according to example 3, starting at the end of the tube where the sample was added;

Figure 20 shows a plot showing the intensity of each plot labelled 13a to 13f from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 21 shows a plot showing the intensity of each plot labelled 14a to 14e from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 22 shows a plot showing the intensity of each plot labelled 15a to 15g from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 23 shows a plot showing the intensity of each plot labelled 16a to 16h from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 24 shows a plot showing the intensity of each plot labelled 17a to 17k from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 25 shows a plot showing the intensity of each plot labelled 18a to 18p from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 26 shows a plot showing the intensity of each plot labelled 19a to 19p from each 0.5 mm interval at a wavelength of 508.4 nm;

Figure 27 shows a plot where the area under the curve in each plot shown in Figures 20 to 26 has been measured and is plotted intensity against concentration in pmoles.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENT

### *Definitions*

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

As used herein, a "wavelength-inspecific beam splitter" is a beam splitter that does not differentiate over a range of wavelengths of excitation radiation or returned radiation to be used in the system. That is, the proportions of the excitation radiation or returned radiation that are reflected and transmitted will not vary significantly over a range of wavelengths of excitation radiation or returned radiation to be used in the system. Preferably, the configuration of the wavelength-inspecific beam splitter is such that the proportions of the excitation radiation or returned radiation that are reflected and transmitted will

not vary significantly over a wavelength range of at least about 350 nm to about 800 nm. In an exemplary embodiment, the proportions of the excitation radiation or returned radiation that are reflected and transmitted will not vary significantly over a wavelength range of at least about 350 nm to about 1000 nm, and in an alternative exemplary embodiment over at least about 350 nm to about 1400 nm.

***Preferred Embodiments***

Figures 1 and 2 show parts of a preferred embodiment analysis system. The analysis system comprises a preferred embodiment microscope section shown in Figure 1, and a preferred embodiment spectrometer section shown in Figure 2.

Referring to Figure 1, the microscope section comprises a sample region 1 for receipt of a sample to be analysed. The sample region may be any sample region that is suitable for holding the sample while enabling the desired analysis to be carried out on the sample. For example, the sample region may comprise a microarray, an optically-transparent surface (e.g. cover-slip or glass slide), or the sample region described below with reference to Figures 3 and 4.

The microscope section has at least one source of excitation radiation 3. In the form shown, a single source of excitation radiation is shown, and can be any suitable type such as a laser that directs laser light into the system for example. The single source of excitation radiation may provide excitation radiation of only a single wavelength. Alternatively, the single source of excitation radiation may provide excitation radiation over a range of wavelengths, which may be used to detect one or more wavelengths, depending on the excitation properties of analytes in the sample. Preferably, the excitation radiation is visible or non-visible light.

In an alternative form, the system may comprise a plurality of sources of excitation radiation. Each of the sources of excitation radiation may be a laser source for example. Any other suitable source(s) of excitation radiation could be used; for example mercury burner(s), xenon discharge lamp(s), or narrow band LED(s). Each of the sources may be configured to provide an excitation radiation with a different wavelength or a different band of wavelengths, so that the system is suitable for detecting multiple analytes in the sample.

The source of excitation radiation 3 is coupled to a beam splitter 13 by a collimating lens 7. An optical fibre 5 directs excitation radiation from the source of excitation radiation to the collimating lens 7, and the collimating lens causes the diverging excitation radiation that is exiting the optical fibre to become parallel as shown to the left of the lens 7 in the figure, and directs that excitation radiation through a field iris 9. The field iris restricts the area of incoming radiation. A focusing lens 11 receives the substantially parallel excitation radiation and causes that to converge, and directs that towards the wavelength-inspecific beam splitter 13.

The wavelength-inspecific beam splitter 13 couples the source of excitation radiation 3 with the sample region 1 to deliver some of the excitation radiation from the source of excitation radiation to a sample to be analysed in the sample region.

The microscope section comprises an objective lens 14 between the beam splitter 13 and the sample region 1. The objective lens may be separated from the sample region by an air gap. Alternatively, the objective lens could be coupled to the sample region by oil, water, or some other liquid, to reduce the number of reflective surfaces. The objective lens may be a magnifying lens, and could be provided with any suitable magnification, such as 1.5x, 4x, 10x, 60x, or any other suitable magnification. The objective lens may be adjustable or replaceable to adjust the magnification.

The configuration of the wavelength-inspecific beam splitter is such that the proportions of the excitation radiation or returned radiation from the sample region that are reflected and transmitted will not vary significantly over a wavelength range of at least about 350 nm to about 800 nm. In an exemplary form, the proportions of the excitation radiation or returned radiation that are reflected and transmitted will not vary significantly over a wavelength range of at least about 350 nm to about 1000 nm, and in an alternative exemplary form over at least about 350 nm to about 1400 nm.

The beam splitter 13 is configured to reflect excitation radiation to the sample region, as shown in Figure 1. The focus F of the focusing lens 11 is beyond the beam splitter 13. The focusing lens 11 and objective lens 14 are configured to focus the excitation radiation on the sample region 1, and the beam splitter 13 turns some of the excitation radiation from the focusing lens to pass through the microscope section objective lens. The beam splitter is configured to reflect only a small proportion of the excitation radiation to the sample in the sample region, and allows the remainder of the excitation radiation to pass through the beam splitter. As can be seen from Figure 1, incoming excitation radiation is reflected from the front surface 13a of the beam splitter, to the section 1a of the sample

region carrying the sample to be analysed. The beam splitter is configured such that its front surface reflects between about 5% and about 15% of the excitation radiation to the sample region (and to a sample therein), and more preferably about 10% of the incoming excitation radiation to the sample region (and to a sample therein).

The majority remainder of the incoming excitation radiation will pass through the front surface 13a of the beam splitter. About the same proportion as is reflected by the front surface will reflect off the rear surface 13b of the beam splitter, and will impact on another section 1b of the sample region. The beam splitter is configured so that excitation radiation being reflected by the rear surface 13b of the beam splitter toward the sample region does not interfere with the excitation radiation being reflected by the front surface 13a of the beam splitter toward the sample region. The beam splitter is configured to have a sufficient thickness, such as at least about 2 mm for example, to prevent or minimise interference. In the preferred form, the beam splitter has a thickness of about 10 mm. Alternatively, the beam splitter may be sufficiently thin that the beams from the back and the front surfaces are both directed to the sample in the sample region. In that configuration, the beam splitter preferably has a thickness of between about 60 and about 150 micrometers, more preferably between about 80 and about 100 micrometers.

The majority of the excitation radiation will pass straight through the beam splitter, and will not be directed to the sample region. In the preferred form, about 90% of the incoming excitation radiation passes through the front surface of the beam splitter, and about 81% of the incoming excitation radiation passes through the rear surface of the beam splitter and is not reflected to the sample region.

The beam splitter can be made of any suitable material. In a preferred embodiment, the beam splitter is made of a glass material, such as BK7 glass for example, which offers substantially linear optical transmission down to about 350 nm from about 2000 nm. The beam splitter could be made of any other suitable material, such as a different type of glass, or a plastic material for example.

The system is preferably configured to provide relative movement between the incoming excitation radiation and the sample region 1, so that the excitation radiation can be directed to selected areas of the sample region or scanned along the sample region. The sample region 1 may be selectively moveable relative to the beam splitter 13, to enable the system to scan the excitation radiation over a desired area of the sample. The sample region will suitably comprise a motorised stage that may be controlled by a suitable controller to enable the system to scan excitation radiation over a desired area

of the sample. The sample region may be movable in one, two, or three dimensions. In a preferred embodiment, the sample region is carried by a Mad City Labs MicroStage-5E micrometer X-Y stage that is controlled by Labview control software. The stage provides for fine positioning of samples, with better than 1 μm accuracy. The stage is incorporated into an Olympus inverted fluorescence microscope. The objective lens 14 used in the preferred embodiment has a x20 magnification, and is an oil immersion objective. The wavelength-inspecific beam splitter 13, laser bank 3 (with 473, 593, 532, 635 nm lasers), a 100 micrometer optical fibre 5, and turning/steering mirror 17 have been added.

The beam splitter 13 is also configured to receive returned radiation from a sample to be analysed in the sample region 1 and to transmit at least some of the returned radiation. The returned radiation comprises residual excitation radiation (such as the bright laser line from the laser source) and radiation to be analysed. At least a major part of the returned radiation from the sample to be analysed that is delivered to the beam splitter, passes through the beam splitter. Preferably, about 85% to about 95% of the returned radiation that is delivered to the beam splitter, passes through the beam splitter. Most preferably, about 90% of the returned radiation that is delivered to the beam splitter, passes through the beam splitter.

The returned radiation that passes through the beam splitter is generally diverging, and a second focusing lens 15 is provided to cause the returned radiation to focus. The returned radiation from the second focusing lens is received by a mirror 17, that directs the returned radiation from the second focusing lens into an entrance 19 of the spectrometer section. The entrance 19 may be an entrance slit or a pin hole entrance. The entrance slit of the spectrometer section is preferably confocal with the sample region 1 of the microscope section. The second focusing lens is configured to focus the returned radiation into the entrance slit or a pin hole entrance.

Turning now to Figure 2, the returned radiation is initially diverging from the entrance slit or pin hole 19 of the spectrometer section, and is received by a collimating mirror 21 to make the returned radiation substantially parallel. The substantially parallel returned radiation is directed by the collimating mirror 21 to a dispersion element 23.

The dispersion element 23 is configured to disperse the returned radiation from the sample to be analysed that has been transmitted by the beam splitter 13. The dispersion element 23 is any suitable form that spatially disperses the returned radiation from the sample to be analysed. For example, the dispersion element could be a prism or a grating. In the preferred form shown in Figure 2, the

dispersion element 23 is a diffraction grating that spatially disperses the returned radiation depending on the wavelengths or wavelength ranges within the returned radiation.

The spatially dispersed returned radiation from the dispersion element 23 is directed to one or more mirrors, and, in the embodiment shown, initially to a plane mirror 25 and then to a further plane mirror 27. A third focusing lens 29 is provided to focus that dispersed returned radiation onto at least one selectively switchable micromirror device (SMD) 35. One or more mirrors may be provided between the third focusing lens 29 and the SMD 35 to direct the returned radiation as required. In the form shown, the dispersed returned radiation is directed from the third focusing lens 29 to a further plane mirror 31, and to a further plane mirror 33, and then to the SMD 35. The dispersed returned radiation from the sample to be analysed is focussed onto the SMD. Preferably, the configuration of the system will be such that the dispersed returned radiation to be analysed is focussed onto the SMD so that the wavelength(s) of returned radiation to be analysed correspond to respective columns or rows of micromirrors of the SMD.

As returned radiation having different wavelengths will be deflected a differing amount by the dispersion element, the dispersion element may be able to be actively turned or rotated to direct the different wavelengths toward the SMD. Alternatively, the system may comprise a plurality of dispersion elements at different angles, to direct the returned wavelengths of the various angles toward the SMD. These alternatives will only be required if the returned radiation to be analysed covers a sufficiently wide wavelength range that it would not otherwise all be directed onto the SMD. Figure 8a shows a diagram of part of an alternative preferred form system that includes a plurality of dispersion elements, and Figure 8b shows an example of the excitation radiation that may be received by the SMD with such a configuration. This alternative configuration is described below.

Returned radiation from the SMD is passed to a detector 43 to detect radiation that is received from the SMD. The SMD removes at least a major part of the residual excitation radiation from the returned radiation that is received by the detector 43. That is, of the returned radiation that is transmitted by the beam splitter and dispersed by the dispersion element, only the radiation to be analysed is passed to the detector, and little or none of the residual excitation radiation is passed to the detector.

The radiation to be analysed from the SMD 35 may be directed to one or more mirrors which deflect the radiation to the detector. In the form shown, the radiation to be analysed is passed through a

fourth focusing lens 37, to a plane mirror 39, to a further plane mirror 41, and on to the detector. The focusing lens is configured to focus the radiation to be analysed onto the detector.

The SMD 35 is preferably a digital micromirror device (DMD) or a digital light processing (DLP) device. Preferably, the SMD has a plurality of columns of micromirrors and a plurality of rows of micromirrors. The SMD is preferably arranged so that either each column or each row of micromirrors, or a plurality of adjacent columns or rows of micromirrors, corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element. Most preferably, the SMD is arranged so that each column, or plurality of adjacent columns, of micromirrors corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element.

The SMD may be controlled by any suitable means, such as via a processing unit that is programmed to control the SMD. The SMD will be controlled to remove at least a major part of the residual excitation radiation from the returned radiation, so that is not passed to the detector. The residual excitation radiation can be removed from the radiation passed to the detector by turning one or more columns of the SMD corresponding to the residual excitation radiation line. Preferably, the SMD is controlled so that the radiation to be analysed is passed to the detector a single column or a plurality of adjacent columns, at a time, so that radiation corresponding to a single wavelength or wavelength range is passed to the detector at a time. However, the SMD may be controlled to remove only the residual excitation radiation, and to direct all of the radiation to be analysed to the detector at once. While that configuration has broad applications, it is particularly useful if the system is used with a sample that photodegrades.

The detector 43 and/or any intervening optical component(s) 37, 39, 41 may be configured relative to the SMD so that when a micromirror of the SMD is not tilted, returned radiation will be passed from that micromirror to the detector. Alternatively the detector 43 and/or any intervening optical component(s) 37, 39, 41 may be configured relative to the SMD so that when a micromirror of the SMD is tilted, returned radiation will be passed from that micromirror to the detector.

The detector 43 may be any suitable type, such as a charge-coupled device (CCD) for example. Other suitable types of detector could be used. For example, an InGaAs detector, silicon detector, or a small photodetector (such as an avalanche photodiode) or point detector could be used.

The radiation to be analysed that is received by the detector 43 will be deconvoluted or decomposed to provide analysis data relating to the received radiation. For example, the radiation received by the detector may be deconvoluted to provide a quantity measurement for to one or more analytes in a sample, where the analytes are labelled directly or indirectly with fluorescence(s). The system will be provided with a processing unit (indicated by reference numeral 47 in Figure 2) that is programmed to provide the desired deconvolution.

In a preferred embodiment, the processing unit will be programmed to enable the system to provide a quantity measurement of at least one, preferably at least two, and more preferably at least five, analytes in a sample from a single excitation radiation. For example, the system as shown is configured to provide a quantity measurement of up to five analytes in a sample from a single laser line.

Rather than having a single source of excitation radiation, the system may have multiple sources of excitation radiation. The system is preferably configured to provide a quantity measurement of up to five analytes in a sample from each source of excitation radiation. As such, if four laser lines are provided, the system is preferably configured to provide a quantity measurement of up to twenty analytes in a sample.

If multiple sources of excitation radiation are provided, the excitation radiation from each of the sources will preferably be delivered to the sample region by the single wavelength-inspecific beam splitter. The excitation radiation from the multiple sources could be delivered to the single beam splitter by any suitable mechanism. For example, where the sources of excitation radiation are a plurality of lasers, dichroic lenses could be provided in the laser bank to deliver the excitation radiation from the multiple sources into the system, via a single optical fibre for example. As an alternative example, each source of excitation radiation could be operatively coupled to a respective optical fibre, with the plurality of optical fibres operatively coupled into a single optical fibre to feed the excitation radiation to the beam splitter. Any other suitable configuration could be used.

The source(s) of excitation radiation will be configured to supply radiation at desired wavelength(s) corresponding to fluorescence(s) of the analyte(s) to be detected. Those wavelengths will generally be within the range of about 400 nm to about 800 nm, possibly within the range of about 400 nm to about 1000 nm, possibly within the range of about 400 nm to about 1400 nm. By way of example only, the excitation radiation wavelength(s) may be one or more of 405 nm, 473 nm, 475 nm, 532 nm, 593 nm, 633 nm, 635nm, 650 nm, or 780 nm.

## Operation of the preferred embodiment system

To analyse a sample, the sample is positioned in the sample region 1. Excitation radiation from the source of excitation radiation is delivered to the sample via the wavelength-inspecific beam splitter 13 to generate returned radiation comprising residual excitation radiation and radiation to be analysed. The returned radiation is received from the dispersion element on the SMD. The SMD is operated to remove at least a major part of the residual excitation radiation from the returned radiation that is passed to the detector but to deliver at least some of the radiation to be analysed to the detector. Preferably, substantially all, and most preferably all, of the residual excitation radiation is removed from the returned radiation that is passed to the detector. However, inconsistencies in the micro-mirrors during manufacture of the SMD, or imperfections in the operation of the SMD, may result in a small proportion of the residual excitation radiation being passed to the detector.

The radiation to be analysed that is received by the detector is deconvoluted or decomposed to provide information relating to the sample. For example, as described in more detail below, the radiation intensity received on the detector may be deconvoluted to provide information on the quantity of one or more analytes in the sample. Alternatively, the system could be used to identify whether there is returned radiation at a wavelength or wavelength range corresponding to one or more specific or target analytes, to determine whether the analyte(s) is/are present in the sample. The system could also be used for other applications.

For example, the system could be used for Raman spectroscopy. By providing a pinhole in the system, the system could be used as a confocal microscope. The system and sample region will be configured according to the purpose the system will be used for an the type of sample to be analysed.

The returned radiation passes from the wavelength-inspecific beam splitter to the detector, without passing through any dichroic beam splitter(s).

## Preferred embodiment quantification method

Figures 3 and 4 show a preferred embodiment analyte quantification method that may be carried out with the system of Figures 1 and 2 or any other suitable system. Steps of a preferred embodiment

method are shown in Figure 3, and Figure 4 shows a sample region for use in the preferred embodiment quantification method.

Referring to Figure 3, the first step involves attaching primary receptors 50 to magnetic beads 51.

The primary receptors 50 may comprise any suitable chemical entity that will bind to the analyte such as probes, ligands or antibodies for example. The receptors may be specific for a target analyte such as monoclonal antibodies or cDNA probes for example that are produced or selected for their specificity to bind to the target analyte. The receptors may alternatively be streptavidin-coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex.

The sample should pass along the length of the sample region with a flow rate that enables adequate binding levels between analyte and primary receptor. If beads are used, the diameter of the bead will be optimised to reflect a number of trade-offs, for example an increase in radius reduces hydraulic resistance to flow but reduces surface area (requires greater sensitivity to detect). Thus, the diameter of the beads affects competing factors: the flow rate of the sample as it passes along the length of the sample region and the surface area per unit volume. Area increases with the square of the diameter (or radius) of the bead and volume increases with the cube of the diameter (or radius) of the bead.

The smaller the bead, the more closely packed they will be and the greater the hydraulic resistance to flow. However, being small increases surface area and makes it more likely that analyte will be bound to a surface as the sample passes over the sample region. If the flow-rate is too high, then not all analyte may be captured. Preferably the flow-rate is selected so that all analyte(s) is/are captured within the length of the sample region that is analysed; that is, the binding of analyte reduces to zero at some point in the region that is analysed. One needs to avoid the situation where hydrostatic pressure on beads (which produces a high flow-rate) compresses the beads, making the hydraulic resistance rise and increasing the pressure still further. There is also an advantage in having sharp peaks (they have well-defined boundaries and make measurement more accurate). In flowing an analyte through a bed of beads, the surface area (stronger signal) should be maximised, flow rate should be optimised, and bead compression should be avoided.

The sample may be passed along the length of the sample region in a stepped or indexed manner. For example, the sample may be moved along the sample region a small distance and held for a length of time, such as two or three hours, before being passed a further distance along the sample region and held for another length of time. The flow rate along the sample region may be increased when the analytes are concentrated using the pre-concentrating method described below.

Magnetic beads 51 are preferably beads or microspheres with a diameter of between about 1 and about 40 micrometers. More preferably the beads have a diameter of between about 1 and about 30 micrometers, even more preferably the beads have a diameter of between about 1 and about 8 micrometers. Most preferably the beads are diamagnetic beads with a diameter of 1.05 micrometers or 5.91 micrometers.

In step 2 the magnetic beads 51 with primary receptors 50 attached are then attached to a surface of the sample region using magnetic attraction, for example using a powerful permanent magnet such as a neodymium magnet. That immobilises the primary receptors 50 to a surface of the sample region.

In alternative embodiments of the invention, the primary receptors 50 may be immobilised on the surface of the sample region in any suitable way, either directly or indirectly.

Where the primary receptors 50 are directly immobilised on a surface of the sample region, the surface may be inherently adsorbent for the receptors or may require chemical activation or modification. Where chemical activation or modification is required, glass surfaces may be treated with a range of silanising reagents that introduce amino, carboxy, sulphydryl and other chemical groups that can be used to attach receptors.

Where the primary receptors are indirectly immobilised on a surface of a sample region, this may be achieved through any suitable method. In a preferred embodiment, beads which attach to the receptors may be attached to the surface of the sample region. The beads may be any suitable beads and may be attached to a surface of the sample region before or after being attached to the receptors. The beads may be magnetic beads 51 as shown in Figure 3 and attached to a surface of the sample region using magnetic attraction, for example using a powerful permanent magnet such as a neodymium magnet. Alternatively, the beads may be covalently attached to the surface. Alternatively, the beads could be immobilised relative to the surface using a matrix as described above.

Step 3 involves preparing labels that are receptor-fluorophore complexes 55 by attaching secondary receptors 56 to fluorophores 57.

The secondary receptors 56 of the receptor-fluorophore complex 55 may comprise any suitable chemical entity that will bind to the target analyte, such as probes, ligands or antibodies, for example. The receptors may be specific for a target analyte. For example, the receptors may be monoclonal antibodies or nucleic acid probes that are produced or selected for their specificity to bind to the target analyte.

The nucleic acid probes can be genomic DNA or cDNA or mRNA, or any RNA-like or DNA-like material, such as peptide nucleic acids, branched DNAs, and the like. The probes can be sense or antisense polynucleotide probes. Where target polynucleotides are double-stranded, the probes may be either sense or antisense strands. Where the target polynucleotides are single-stranded, the probes are complementary single strands.

The probes can be prepared by a variety of synthetic or enzymatic schemes, which are well known in the art. The probes can be synthesized, in whole or in part, using chemical methods well known in the art (Caruthers et al., *Nucleic Acids Res., Symp. Ser.,* 215-233 (1980)). Alternatively, the probes can be generated, in whole or in part, enzymatically.

Nucleotide analogs can be incorporated into probes by methods well known in the art. The only requirement is that the incorporated nucleotide analog must serve to base pair with target polynucleotide sequences. For example, certain guanine nucleotides can be substituted with hypoxanthine, which base pairs with cytosine residues. However, these base pairs are less stable than those between guanine and cytosine. Alternatively, adenine nucleotides can be substituted with 2,6-diaminopurine, which can form stronger base pairs than those between adenine and thymidine.

Additionally, the probes can include nucleotides that have been derivatized chemically or enzymatically. Typical chemical modifications include derivatization with acyl, alkyl, aryl or amino groups.
The probes can be immobilized on a substrate. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which the polynucleotide probes are bound. Preferably, the substrates are optically transparent.

Furthermore, the probes do not have to be directly bound to the substrate, but rather can be bound to the substrate through a linker group. The linker groups are typically about 6 to 50 atoms long to provide exposure to the attached probe. Preferred linker groups include ethylene glycol oligomers, diamines, diacids and the like. Reactive groups on the substrate surface react with one of the terminal portions of the linker to bind the linker to the substrate. The other terminal portion of the linker is then functionalized for binding the probe.

The probes can be attached to a substrate by dispensing reagents for probe synthesis on the substrate surface or by dispensing preformed DNA fragments or clones on the substrate surface. Typical dispensers include a micropipette delivering solution to the substrate with a robotic system to control the position of the micropipette with respect to the substrate. There can be a multiplicity of dispensers so that reagents can be delivered to the reaction regions simultaneously.

The receptors may alternatively be streptavidin coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex. The analyte(s) may be labelled either before or after passing the sample along the length of the sample region.

Where the analyte(s) is/are labelled before passing the sample along the length of the sample region, labels may be added to the sample and the sample allowed to incubate for a period sufficient to ensure binding. For example, in step 4 of Figure 3, the receptor-fluorophore complexes 55 (labels) are then incubated with analyte 60 so that the analyte 60 binds to the receptor-fluorophore complexes 55 creating analyte-receptor-fluorophore complexes 61 (or labelled analytes).

When the analyte(s) is/are labelled after the sample has been passed along the length of the sample region, the labels may be passed along the length of the sample region with a flow rate that allows for the labels to bind to the analyte.

Preferably the analyte 60 is protein, peptide, DNA, RNA, carbohydrate or lipid. Where the sample is a biological sample, the analyte may be one or more foreign bodies such as drug(s). In alternative embodiments where more than one analyte is being quantified, the analytes are preferably protein(s), peptide(s), DNA, RNA, carbohydrates(s), lipid(s), foreign bodies such as drug(s), or mixtures thereof.

In step 5, the analyte-receptor-fluorophore complexes 55 are then passed along a length of the sample region with a flow-rate that allows adequate binding levels between analyte 60 and secondary receptor 56. As discussed above, where beads are used, the size of the bead can be selected so as to influence the flow-rate of the sample as it passes over the sample region.

In alternative embodiments of the invention, the analyte(s) in the sample may be labelled in any suitable way where the analysis system can quantify the analyte(s) from information received from the labels.

The analyte(s) may be fluorescently labelled as shown in Figure 3 or otherwise fluorescently-labelled, labelled using chemically-driven light-emitting systems that are non-fluorescent such as luciferases for example, or labelled using non-electromagnetic radiation such as magnetism for example. In alternative embodiments the analyte(s) may be enzymes that generate fluorescent products such as modified ELISAs for example or aptamers such as DNA or RNA molecules that quench or unquench when complementary DNA or RNA strands bind or the aptamers fold or unfold.

Fluorescent labels that may be used include, but are not limited to, the following:

| | **Fluorophore** | **Excitory Peak (nm)** | **Emission Peak (nm)** |
|---|---|---|---|
| Group One 473 nm | FITC (Flouroscein) | 494 | 518 |
| | PE | 488 | 575 |
| | Texas Red | 488 | 615 |
| | Cy7 | 488 | 760 |
| | Alexa Fluor 500 | 503 | 525 |
| Group Two 532 nm | Eosin | 524 | 544 |
| | Alexa Fluor 532 | 532 | 554 |
| | Alexa Fluor 555 | 555 | 565 |
| | Alexa Fluor 568 | 578 | 603 |
| | Alexa Fluor 594 | 590 | 617 |
| Group Three 633 nm | Alexa Fluor 610 | 612 | 628 |
| | Alexa Fluor 633 | 632 | 647 |
| | Alexa Fluor 647 | 633 | 668 |
| | Alexa Fluor 750 | 633 | 775 |
| | DyLight 633 | 638 | 658 |
| Group Four 650 nm | DyLight 649 | 646 | 674 |
| | Alexa Fluor 660 | 663 | 690 |
| | Alexa Fluor 680 | 679 | 702 |
| | DyLight 680 | 682 | 715 |
| | Alexa Fluor 700 | 702 | 723 |

These fluorophores are available from various suppliers including Dylight, Invitrogen, and Bioscience.

While the excitory peaks differ for some of the fluorophores in each group, the fluorophores in a group will all be caused to fluoresce by a laser having a wavelength listed in the left column for the group. Some of the fluorophores may also be caused to fluoresce by the laser from the adjacent group.

Preferably, the labels are selected to minimise the number of excitation wavelengths to be used. In a preferred embodiment 20 analytes are used with five or more fluorophores excited by the same wavelength, but emitting at five different wavelengths.

The fluorescently labelled receptors may be prepared by methods known to those skilled in the art. Suitable fluorescently labelled receptors include fluorescently labelled antibodies to antibodies of species such as rat, mouse, rabbit, guinea pig and chicken.

In another embodiment antibodies to the analytes may be labelled with the fluorophores for use in the method shown in figure 3.

As shown in step 6, the fluorophores 57 receive excitation radiation 80 and return at least some of that radiation to a detector of the analysis system. Where no analyte 60 is present, there will be no returned radiation at the particular wavelength or wavelength range corresponding to the fluorescence, and therefore a "negative" result.

The analysis system may be any suitable system that can provide an intensity for a particular wavelength that can be deconvoluted to quantify analytes in a sample. Preferably the analysis system is the analysis system described above with reference to Figures 1 and 2.

The analysis system may be any suitable system comprising a detector that can be used to quantify the target analyte in the sample. When the analyte(s) is/are labelled fluorescently, the analysis system preferably comprises at least one source of excitation radiation. Preferably, the excitation radiation is directed into the sample region in a direction transverse to the length of the sample region, and is moved along at least part of the length of the sample region comprising the immobilised receptors. The excitation radiation may also be moved across at least part of the width of the sample region comprising the immobilised receptors. The detector receives at least some of the returned radiation.

As shown in steps 7 and 8 of the preferred embodiment analyte quantification method, the analysis system collects spectra from received radiation from sections of the tube and the analyte is quantified by deconvoluting the spectra.

In a preferred embodiment, the number of analytes bound to receptors generally reduces along at least part of the length of the sample region comprising receptors as the number of unbound analytes in the sample reduces due to binding of the analyte to the receptors. More analyte will bind to receptor-bearing beads at the first end of the sample region (where the sample enters) than further away from the first end. Accordingly, the intensity of returned radiation relating to the fluorescence of the analyte that is delivered to the detector will generally reduce along the length of the sample region from the first end as the number of unbound analyte(s) in the sample reduces due to binding with the immobilised receptors. Beyond a certain point, there may be no binding, and the excitation radiation will not generate any returned radiation. This creates an intensity profile that changes along the length of the sample region, and this information can be used to quantify the level of analyte in the sample. Where the quantity of more than one analyte is being analysed, different analytes may travel to different points along the sample region.

The intensity of the returned radiation may not reduce along the entire length of the sample region. For example, depending on the affinity between the analyte(s) and immobilised receptors, the analyte(s) may initially saturate the receptors thereby providing a generally constant intensity of returned radiation along a part of the sample region before the number of unbound analyte(s) in the sample reduces so that a corresponding reduction in the intensity of the returned radiation is observed.

Referring to Figure 4, the sample region 1 may be any sample region that is suitable for receipt of the sample while the method is performed. For example the sample region may comprise any suitable chamber, such as a tube, capillary, column or cuvette for example. The chamber may be open at one or both ends. A majority of the sample, other than the analyte that has bound to the receptors, may pass through and exit the sample region. In the preferred form, the sample region is a capillary tube having a rectangular cross-section with a height of 50 micrometers, a width of 500 micrometers, and a length of 10 mm. A square or rectangular tube is particularly suitable if it is desired to carry out analysis near the edges of the sample, as the relatively sharp corners will result in less optical distortion than a curved tube. If the analysis is to be carried out largely along the centre of the tube, a curved edge tube could be used. The tube could have any other suitable dimensions or configuration.

The sample region may be reusable or disposable.

In Figure 4, the preferred embodiment method comprises analysing a sample to quantify two analytes 70a and 70b in the sample.

Primary receptors 50a and 50b are indirectly immobilised on a surface of the sample region through attachment to magnetic beads 51. In alternative embodiments the receptors may be otherwise immobilised on a surface of the sample region, either directly or indirectly.

The primary receptors 50a and 50b may comprise any suitable chemical entity that will bind to the analyte such as probes, ligands or antibodies for example. The primary receptors 50a and 50b may be specific for a target analyte such as monoclonal antibodies or cDNA probes for example that are produced or selected for their specificity to bind to the target analyte. The receptors may alternatively be streptavidin-coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex.

The magnetic beads 51 may be any suitable beads including those discussed with reference to Figure 3.

The receptors are immobilised along at least part of the length of the sample region 1, such as 1 mm in length and the entire height and width of the sample region 1 for example.

Alternatively the method may comprise analysing a sample to quantify one analyte in the sample. Alternatively the method comprises analysing a sample to quantify each of several analytes in the sample. Preferably, the method comprises analysing a sample to quantify each of at least two, three, four, more preferably at least five, and most preferably at least twenty analytes in a sample.

Where the method involves quantification of more than one analyte, the method must allow for different analytes to be identified. This may be achieved in a number of ways.

In a preferred embodiment, the method may allow for the quantification of different analytes where the labels are specific for target analytes. For example, if the analytes are labelled through receptor-fluorophore complexes, specific receptors may be attached to specific fluorophores. Where the quantity of two analytes is desired, a first group of receptors specific for the first analyte is attached to

fluorophores that return radiation at a first wavelength or wavelength band and a second group of receptors specific for the second analyte are attached to a second group of fluorophores that return radiation at a second wavelength or wavelength band. In this embodiment the analysis system can distinguish between different analytes by deconvoluting the returned radiation with reference to different wavelengths or wavelength bands each corresponding to a particular fluorescence that is associated with a particular analyte in the sample.

In an alternative embodiment, the immobilised receptors are specific for a target analyte and the analysis system can detect the difference between different receptors. For example, if the receptors are indirectly immobilised on a surface of the sample region through beads, and the quantity of two analytes is desired, then receptors specific to the first analyte may be attached to a group of beads of a first colour, and receptors specific to the second analyte may be attached to a group of beads of a second colour. Where the quantity of three analytes is desired, the third group of receptors specific for the third analyte may be attached to a group of beads of a third colour. In this embodiment the analysis system can distinguish between different analytes by deconvoluting the returned radiation with reference to the different groups of coloured beads.

Where reference is made herein to groups of beads of different colours, each bead of one group may have a different emitted spectrum or colour to each bead of another group. Alternatively, each bead of one group may have a different combination of colours to each bead of another group.

In a most preferred embodiment, a combination of both immobilised receptors attached to coloured beads and fluorophores of different wavelengths are used. The immobilised receptors and receptor-fluorophore complexes have a certain level of specificity that allows for a large number of different analytes to be analysed in the same sample.

The analytes 70a and 70b may be protein(s), peptide(s), DNA, RNA, carbohydrates(s), lipid(s), or mixtures thereof. Where the sample is a biological sample, the analyte(s) may be one or more foreign bodies such as drug(s).

The analytes 70a and 70b are fluorescently labelled using receptor-fluorophore complexes 55 comprising complexes between secondary receptors and fluorophores. The fluorophores 57 receive the excitation radiation 80 and return at least some of that radiation as returned radiation 81 to a

detector. Where no analyte is present, there will be no returned radiation at the particular wavelength or wavelength range corresponding to the fluorescence, and therefore a "negative" result.

The excitation radiation 80 is directed into the sample region in a direction transverse to the length of the sample region, and is moved along the length of the sample region to which the receptors are immobilised. Additionally, the excitation radiation may be moved across the sample region, to obtain a two dimensional analysis of the sample in the region. That can be achieved by moving the sample region 1 relative to the beam splitter 13, such as via the motorised stage for example. From the returned radiation 81, the analyte(s) is/are quantified.

As shown in Figure 4, the sample has been passed along the length of the sample region comprising receptors, whereby the analytes 70a and 70b have bound to receptors 50a and 50b, wherein the number of analytes bound to receptors has generally reduced along the length of the sample region as the number of unbound analytes in the sample reduces due to binding of the analytes to the receptors. In Figure 4, the number of the first analyte 70a is greater than the number of the second analyte 70b. The analysis system, by deconvoluting the spectra of returned radiation, can quantify the analytes 70a and 70b.

**Preferred method of preparing a sample to quantify analyte in the sample**

**Sample preparation**

The sample may be any biological or non-biological sample where knowledge of the quantity of at least one specific analyte is desired. When the sample is a biological sample, it may be human or non-human and it may be selected from any biological material including blood, saliva, semen, vaginal secretion, urine, faecal material, for example, or cells including embryonic cells for example, or organs or parts of organs for example, or mixtures thereof. Alternatively the sample may be taken from soil, water or air, for example.

As a preliminary step it may be necessary to process the sample first.

For example, the sample may contain debris or other unwanted material that may interfere with the ability of the analytes to bind with the receptors or block the sample region. Any debris or other

unwanted material in the sample may be removed by filtering the sample through a series of filters for example or by centrifuge.

The sample may also contain intact cells where the analyte is contained within the cell and it is necessary to lyse the cells and release the contents so that any analyte is free to bind with a receptor. Intact cells may be broken up using any known technique. For example, a non-ionic detergent may be used to dissolve or disrupt cellular membranes and solubilise membrane proteins and release intracellular protein components. Suitable non-ionic and ionic detergents include polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether (TRITON X-100). In some instances a non-denaturing detergent may be suitable, for example, cetyl trimethyl ammonium bromide.

The sample may also contain intact cells that are to be removed before the fluid of the sample is analysed. For example, blood samples may be treated to remove whole cells by, for example, coagulation, filtering or centrifugation.

It may also be necessary to prepare the sample in other ways. For example, cDNA may be prepared from mRNA for quantification, so that the cDNA is the analyte in the sample for analysis.

DNA or RNA can be isolated from the sample according to any of a number of methods well known in the art. For example, methods of purification of nucleic acids are described in Tijssen; Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with nucleic acid probes Part 1: Theory and Nucleic acid preparation, Elsevier, New York, N.Y. 1993, as well as in Maniatis, T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual 1989, herein incorporated by reference.

**Preferred method of pre-concentrating analytes of interest**

The sample may be crude and contain a number of different types of analytes and debris that are not of interest. This can cause the rate of binding of the analyte of interest to the receptor/ligand to be very slow. Additionally, the binding of some analytes to some receptors, particularly the binding of an analyte to an antibody, is also slow because of the steric constraints that exist when two complex molecules bind. Therefore, it is desirable to use a pre-concentrating method to concentrate the analyte of interest so that the rate of binding of the analyte to its receptor is increased.

When the analyte of interest is a nucleic acid the nucleic acid can be isolated from the sample according to any of a number of methods well known in the art. For example, methods of purification of nucleic acids are described in Tijssen; Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with nucleic acid probes Part 1: Theory and Nucleic acid preparation, Elsevier, New York, N.Y. 1993, as well as in Maniatis, T., Fritsch, E. F. and Sambrook, J., Molecular Cloning Manual 1989.

When the analyte of interest is a protein or group of proteins, the pre-concentrating step may include isolation from a host cell expressing the protein and purification according to standard procedures of the art, including ammonium sulfate precipitation, column chromatography (*e.g.*, ion exchange, gel filtration, affinity chromatography, *etc.*) and electrophoresis (see generally "Enzyme Purification and Related Techniques", (1971) *Methods in Enzymology*, 22:233-577 incorporated herein by reference).

Preferably the pre-concentrating step concentrates the analytes of interest into a small volume prior to introducing this small volume to the sample region containing the receptors for binding the analytes.

Preferably, ion exchangers are used for the pre-concentrating step to capture analytes that have a net charge opposite to the ion exchanger.

In a preferred method a large volume of sample is contacted with ion exchanger under conditions where the analytes bind. The analytes are then eluted from the ion exchanger in a small volume. The elution can be brought about by, for example, increasing the salt concentration or changing the pH.

Ion- exchangers useful in the present invention may have an ionic group linked to a rigid or solid support. The rigid or solid support can be, for example, membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries.

The ion-exchangers may include the use of a chemically labile linker that joins an ionic group to the solid support. The chemically labile linker can be cleaved, which results in the liberation of the analyte of interest. Many chemically cleavable linkages are known to those skilled in the art; for example, linkers such as, but not limited to, disulphide linkage, which can be cleaved using mercaptans and cis diols, which may be cleaved using periodate.

**Receptors immobilised along a length of the sample region**

The receptors immobilised along a length of the sample region may comprise any suitable chemical entity that will bind to the analyte such as probes, ligands or antibodies for example. The receptors may be specific for a target analyte such as monoclonal antibodies or cDNA probes for example that are produced or selected for their specificity to bind to the target analyte.

The receptors may alternatively be streptavidin-coated molecules where biotin is attached to the target analyte or alternatively the receptors may be biotin or molecules with biotin attached where the analyte is bound to streptavidin and the receptors bind to the analyte through a streptavidin-biotin complex.

For example, where the quantity of one or more mRNA is desired, the mRNA in the sample can be converted to cDNAs with biotin-oligod-T primers and bound to streptavidin-derivatised glass. Antisense DNA probes carrying fluorescent labels can be hybridised to the streptavidin-biotin-bound cDNAs.

In another variant of mRNA detection, the receptors are DNA probes that are attached to the glass (using the streptavidin-biotin system or some other appropriate chemistry) and the mRNAs are hybridised to the receptors or converted to cDNAs and hybridised to receptors.

The receptors may alternatively be any other suitable receptor.

**Passing the sample along a length of the sample region**

In order to pass the sample along a length of the sample region, the sample may be poured or pumped (such as via a micro-pump for example) into one end of the sample region and is forced to travel along the length of the sample region by gravity or some other means. Alternatively the sample region may be centrifuged to cause the sample to travel along the length of the sample region comprising receptors. This may or may not be achieved through gravity. Alternatively the sample may be drawn into the chamber and along the sample region using capillary action.

The sample suitably passes along the length of the sample region starting at a first end. The flow rate of the sample will be selected to ensure that the analyte attachment is not overly attenuated as it passes down the column of analyte binds to receptors when they meet. The flow rate may be manipulated by altering the viscosity of the sample or the size of the beads or the hydrostatic pressure (increase by

increasing pump speed or centrifugal rate). Where the receptor is bound to a bead, the beads act to increase the surface to volume ratio and provide hydrostatic resistance to slow the flow rate and ensure adequate binding levels between analyte and receptor.

The sample may be passed along a length of the sample region in a stepped or indexed manner if required.

In a preferred embodiment, the number of analytes bound to receptors generally reduces along at least part of the length of the sample region comprising receptors as the number of unbound analytes in the sample reduces due to binding of the analyte to the receptors. More analyte will bind to receptor-bearing beads at the first end of the sample region than further away from the first end. Accordingly, the intensity of returned radiation relating to the fluorescence of the analyte that is delivered to the detector will generally rise sharply to begin with and then reduce along the length of the sample region from the first end as the number of unbound analyte(s) in the sample reduces due to binding with the immobilised receptors. Beyond a certain point, there may be no binding, and the excitation radiation will not generate any returned radiation. This creates an intensity profile that changes along the length of the sample region, and this information can be used to quantify the level of analyte in the sample, for example, by integrating the area under the curve or the volume under envelope. Where the quantity of more than one analyte is being analysed, different analytes may travel to different points along the sample region.

**Quantification of analyte in sample using radiation intensity data**

Figure 5 shows a schematic view of the sample region when used to quantify analyte in the sample. A portion 1' of the length of the sample region tube contains immobilised receptors, as discussed above. The receptors will suitably be immobilised along that portion 1' of the length, as well as over the full height and width of the tube in that portion of the length. The excitation radiation 80 is directed into the sample region in a direction transverse to the length thereof. In one embodiment, these positions represent adjacent non-overlapping slit widths, but other configurations are possible. The portion 1' of the sample region can be split into a finite number of sub-sections, each corresponding in width to the width of the excitation radiation beam.

The excitation radiation is scanned along at least part of the length of the portion 1'. This is achieved a step-wise manner, so the radiation initially impacts on region 101a, then region 101b, then region 101c,

etc. The returned radiation is passed through the system of Figure 1 and 2, and detected on the detector.

Figure 6a shows an example plot of the radiation that may be received by the detector 43 of Figure 2 for point 101a of the sample region. For the given incoming excitation radiation 80, if there are analytes with differing fluorescences that are triggered by the radiation, the returned radiation to be anlaysed will include peaks 103, 105 corresponding to those radiations. Figure 6b shows an example plot of the radiation that may be received by the detector for point 101b of the sample region. Again, the signal includes fluorescences that are triggered by the incoming excitation radiation, so the returned radiation to be anlysed includes peaks 103', 105'.

For each point 101a, 101b, 101c, the returned radiation to be analysed will be delivered to the detector by the SMD. The returned radiation for a given point may be delivered to the detector in its entirety, or the SMD could be used to remove selected parts of the returned radiation. For example, the wavelength or wavelength band corresponding to each target analyte may be delivered to the detector, one at a time.

As the quantity of target analytes that attach to the beads generally reduces along a direction of the length of portion 1', the intensity of the peaks 103', 105' may be lower than 103, 105. The system is operable to extract the data relating to a specific wavelength or wavelength region along the portion 1', to extract data relating to a target analyte. The system may be programmed so that radiation received by the detector is deconvoluted to provide data relating to specific wavelengths or wavelength ranges, and thereby target analytes. Alternatively, for each point 101a, 101b, etc in the portion 1' of the tube, the SMD could be operated to deliver a wavelength or wavelength range at a time to the detector. The system would store the data and then combine that to provide data relating to each desired wavelength or wavelength range, and thereby each target analyte.

Figure 7a shows an example plot of returned radiation intensity versus point 101a, 101b, etc that is received by the detector for a specific wavelength. This may have been extracted from the overall excitation radiation using either method mentioned above. Each analyte in the sample can be quantified by calculating the area under the intensity curve for the particular wavelength or wavelength range associated with the target analyte. That is the shaded area shown in Figure 7a. The system will be programmed to perform that calculation based on the returned radiation to be analysed that is

received by the detector. That area corresponds to the total intensity of returned radiation received by the detector for the specific wavelength or wavelength band.

The system can perform the same steps for each analyte; that is, extract the intensity of the relevant wavelength or wavelength band in question from the returned radiation, establish the total intensity of returned radiation at the relevant wavelength or wavelength band from the intensity for that wavelength or wavelength band for each point 101a, 101b where there is returned radiation at the relevant wavelength, and quantify the analyte.

The area under the plot of Figure 7a representing the total intensity of returned radiation, is proportional to the quantity of the analyte in the sample. The proportional relationship may or may not be a linear relationship. To calculate the actual quantity, the total intensity may be compared to a total intensity obtained by carrying out the same process for a sample containing a known quantity of an analyte, or alternatively the system could be programmed with relevant factors relating to the analytes in question to establish the actual quantity. The system may be programmed with factors that have been established based on column (sample region) parameters, reagent parameters, etc. Provided the columns are manufactured accurately, and the samples are prepared and analysed using techniques with high repeatability, the programmed factors will enable the system to provide automatically an accurate quantification of the analyte(s) in the sample.

As another alternative, a known quantity of another analyte could be added to the sample, and the radiation intensity for the known analyte could be compared to the radiation intensity for the analyte(s) in question, to obtain quantities of these analytes.

When the volume of sample that is passed along the length of the sample region is known, the quantity of analyte determined by the system can be divided by that volume to obtain a concentration of the analyte in the sample. The system may be programmed with the volume to calculate automatically the concentration of each analyte(s).

In some cases, it may be desirable to obtain a reading both along at least a portion of the length and across at least a portion of the width of the sample region. That can be achieved by, for each point 101a, 101b, 101c etc, additionally taking a reading at each of a number of points across the width of the sample region. Figure 7b shows an intensity curve for such a configuration. The volume under the curve will again be a representation of total intensity of radiation at the relevant wavelength or

wavelength band received by the detector, and will be proportional to the quantity of the analyte in the sample. Again, the system will be configured as described above, to deconvolute the data relating to each wavelength or wavelength band corresponding to a target analyte, and to calculate the quantity of the analyte from that data.

The methods described above provides an output of intensity relative to position along the sample for a specific wavelength or wavelength range which corresponds to a specific fluorescently labelled analyte. That output can be used to quantify the target analyte in the sample, by calculating the total intensity of radiation received by the detector that corresponds to a particular wavelength or wavelength range.

It will be appreciated that the system could be operated in different ways to extract the required data. When multiple analytes are to be detected, each of which has a specific wavelength or wavelength range, the system may take all required readings at each point 101a, 101b, 101c, before moving on to the next point. For example, a first source of excitation radiation may be triggered at point 101a, and the readings from one or more associated analytes could be obtained. Then, the second source of excitation radiation may be triggered at point 101b, and the readings from one or more associated analytes could be obtained, and so on, before moving on to point 101b and repeating the process.

Alternatively, one or more analytes may be analysed at all relevant points 101a, 101b, 101c, etc, before analysing one or more different analytes at all relevant points 101a, 101b, 101c, and so on.

If a point detector is used, the SMD will be used to direct returned radiation to the detector only one wavelength or wavelength band at a time

It is preferred that the method is carried out using the apparatus of Figure 1 and 2, as that enables at least a major part of the residual excitation radiation to readily be excluded from the radiation sent to the detector. The method could be carried out with any other suitable apparatus that enables radiation intensity to be obtained for each relevant wavelength or wavelength band.

The system may also be used where the analyte is not fluorescently labelled as described above. With such an alternative, there would be no residual excitation radiation to be removed from the signal sent to the detector. However, the apparatus of Figures 1 and 2 would still be useful for the analysis as it enables the different wavelengths or wavelength bands to be readily distinguished.

**Alternative preferred embodiment system**

Figure 8 shows part of an alternative preferred embodiment system that includes a plurality of dispersion elements. Unless described below, the features and operation should be considered the same as for the above figures, and like numerals indicate like parts with the addition of 1000. In this embodiment, the dispersion elements 1023 comprise a plurality of dispersion elements 1023a, 1023b, 1023c, 1023d that are oriented at different angles, to direct the returned wavelengths of the various angles toward the SMD. The system comprises a plurality of dispersion elements 1023a, 1023b, 1023c, 1023d in a stack, with each dispersion element oriented at a different angle relative to the other dispersion elements.

The returned radiation impacts on the dispersion elements. As the returned radiation will deflect a different amount depending on its wavelength, the dispersion elements are angled relative to each other so that all relevant returned radiation is directed to the SMD. Figure 8b shows an example image of radiation received on the SMD. As the dispersion elements are configured in a stack, the system can direct the radiation of differing wavelengths simultaneously to the SMD surface as shown. Radiation directed by element 1023a will be positioned above the radiation directed by element 1023b, and so on. Where each wavelength band has residual excitation radiation, the SMD will be used to prevent the excitation radiation from being directed to the detector, or to at least minimise the amount of excitation radiation that is passed to the detector. If the differing fluorescences triggered by a single excitation radiation source cover a sufficiently wide wavelength range, there may only be one residual excitation radiation band to remove.

The four groups of resulting excitation radiation can be simultaneously directed to the detector and analysed as above, or the SMD could be used to direct the wavelengths one at a time as outlined above. This will be repeated for each point in the portion 1' of the sample region of which analysis is required.

The system will be configured so that the excitation radiation directed to the stack of dispersion elements is configured as a series of collimated beams, or so that the beam being received by the stack is sufficiently deep that it covers the plurality of dispersion elements. The stack may have more or less than four elements.

**Further alternative preferred embodiment system**

Figure 12 shows an alternative preferred embodiment spectrometer section for an alternative preferred embodiment system. In the alternative preferred embodiment system, the spectrometer section replaces that shown in Figure 2. Unless described below, the features and function should be considered the same as for the above figures, and like numbers indicate like parts with the addition of 2000 to the numbers used in Figure 2. The spectrometer section shown in Figure 12 is used with the microscope section of Figure 1.

In this alternative preferred embodiment, several of the components 21, 23, 25, 27, 29, 31, and 33 are replaced by a single element, namely a concave holographic grating2023. One suitable grating is an NT47-565 600 groove/mm concave holographic grating available from Edmund optics. The concave holographic grating acts as the dispersion element for this embodiment. The concave holographic grating both diffracts and focuses the returned radiation from the entrance slit or pin hole 2019 of the spectrometer section. One suitable entrance slit is a VS100/M adjustable slit from Thorlabs.

The concave holographic grating 2023 is configured to disperse the returned radiation from the sample to be analysed that has been transmitted by the beam splitter 13. The concave holographic grating 2023 spatially disperses the returned radiation depending on the wavelengths or wavelength ranges within the returned radiation.

The spatially dispersed returned radiation from the concave holographic grating2023 is directed to one or more mirrors, and, in the embodiment shown, initially to a plane mirror M1 and then to a further plane mirror M2, and onto the selectively switchable micromirror device (SMD) 2035. The spacially dispersed returned radiation is focused onto the SMD by the concave holographic grating. Suitable mirrors for M1 and M2 are 2" diameter steering mirrors with broadband visible dielectric E02 coating from Thorlabs. One suitable SMD 2035 is a DMD from Texas Instruments.

Depending on the configuration of the spectrometer section, plane mirrors M1, M2 could be dispensed with.

Preferably, the configuration of the system will be such that the dispersed returned radiation to be analysed is focussed onto the SMD so that the wavelength(s) of returned radiation to be analysed correspond to respective columns or rows of micromirrors of the SMD.

Returned radiation from the SMD is passed to a detector 2043 to detect radiation that is received from the SMD, by a focusing lens 2037. The SMD removes at least a major part of the residual excitation radiation from the returned radiation that is received by the detector 2043. That is, of the returned radiation that is transmitted by the beam splitter and dispersed by the concave holographic grating, only the radiation to be analysed is passed to the detector, and none or little of the residual excitation radiation is passed to the detector. The focusing lens 2037 is configured to focus the radiation to be analysed onto the detector.

One suitable focusing lens 2037 is a Hastings triplet image relay lens, f= 120mm. One suitable detector is a 1340x100 pixel TEC CCD array, available from Princeton Instruments.

By using fewer components in the spectrometer section, the spectrometer section can be more compact, can operate with higher efficiency, and will likely cost less to manufacture. Additionally, the configuration shown in Figure 12 should result in lower light scattering due to reduced optical surfaces. The concave holographic grating can provide a higher line count and greater dispersion than the grating used in the embodiment of Figure 2. Therefore, the sensor 2043 may need to be physically larger than that used in the first embodiment, or a plurality of sensors may be used.

It is preferable to maximise (within reason) the path length between the dispersion element 2023 and the SMD 2035, to reduce the amount of scattered radiation that is transferred to the SMD 2035.

The system could incorporate a plurality of stacked concave holographic gratings 2023 on different angles, such as described in relation to Figure 8a

## EXAMPLES

### EXAMPLE 1

Strepavadin-coated magnetic beads with a diameter of 1.05 micrometers were obtained from Dynal.

The beads were mixed with a highly concentrated commercially available biotin-4-fluorescein (Molecular Probes) in aqueous solution, and incubated in the dark overnight in a fridge (0-4 degrees C). The objective of this step was to get each bead fully coated with biotin complex.

The bead-biotin-fluorophore complexes were then washed with 1ml phosphate buffered saline three times.

Approximately 100 microlitres of the bead slurry was taken and a commercially available magnetic bead extractor used to extract the bead-biotin-fluorophore complexes from the slurry.

A small amount (approximately 10 microlitres) of the bead-biotin-fluorophore complexes was introduced into a rectangular Vitrocom glass tube having internal dimensions 50 micrometers by 500 micrometers by 30-50 micrometers in length).

A small neodymium magnet (5 micrometers by 1 micrometers) was held close to the middle of the tube. As the slurry travelled along the tube due to capillary action the magnetic beads were arrested by the magnet.

The tube was then placed under a microscope and the sample excited with a 532 nm laser and the intensity of the fluoresced radiation measured.

Figure 9 shows the intensity measurement obtained from the sample when measured by the CCD in the system.

This experiment demonstrated that we could arrest the beads and measure the intensity of the fluorescence from the sample.

**EXAMPLE 2**

Strepavadin-coated magnetic beads with a diameter of 1.05 micrometers were obtained from Dynal.

A first group of beads were mixed with an aqueous solution Alexa Fluor 514 carboxylic acid, succinimidyl ester (Molecular Probes) that had been biotinylated using EZ-Link Amino-PEO$_3$-Biotin (Pierce), and incubated in the dark overnight in a fridge (0-4 degrees C). These beads were referred to as Beads 2.

A second group of beads were mixed with an aqueous solution Alexa Fluor 555 carboxylic acid, succinimidyl ester (Molecular Probes) that had been biotinylated using EZ-Link Amino-PEO$_3$-Biotin (Pierce) as for the first group. These beads were referred to as Beads 5.

The objective of this incubation step was to get each bead fully coated with Alexa Fluor/biotin complex.

The bead-biotin-fluorophore complexes were then washed with five times in 1ml phosphate buffered saline. The wash steps were carried out using a Magnetic bead concentrator (Dynal) and the washed beads re-suspended in a final volume of 100 microlitres.

Ten microlitres of the two groups of beads were mixed together. Ten microlitres of this mixture was then immediately introduced into a Vitrocom glass tube. This was achieved as described above. That is, a small neodymium magnet (5 micrometers by 1 micrometers) was held close to the middle of the tube. As the slurry travelled along the tube due to capillary action the magnetic beads were arrested by the magnet.

The tube was then placed under a microscope and the sample excited with a 473 nm laser at 21 sections along the tube and the intensity of the fluoresced radiation measured.

The results are shown in Figures 10 and 11. The spectra was decomposed at each section into the individual components. The area under each decomposed component can be calculated, effectively giving a concentration at each point. The area can be plotted as a function of position to give the concentration of the entire sample. The concentration of the dye is directly proportional to the area under the curve for the dye of interest. The area is proportional to the curve peak height (for that dye). As we are examining ratios of concentration, the heights of the decomposed signal were measured.

The plots in Figure 10 are each representative of the readings at a single position along the sample region; namely 500 micrometers. In the third plot of Figure 10, a small peak can be seen at about 473 nm. That represents the small amount of residual excitation radiation that was not removed by the SMD, due to inconsistencies in the SMD's manufacture or operation. This peak can be useful for calibrating the system, as the wavelength corresponding to that peak is known.

The small peak seen at the excitation wavelength is due to imperfections in the micro mirror surfaces as mentioned in the paragraph above. However, the unwanted excitation light which overlays part of the main convolved spectra signal is due to imperfect imaging of the spectrometer slit upon the SMD surface. This arises through scattering of the slit light as it propagates via the many components starting at the microscope and finishing at the mirror immediately before the SMD array. Lengthening path lengths can minimise the effects of such light scattering.

Figure 11 shows the relative signal strengths of each of bead 2 and 5, at points along the length of the sample region. In this example, the beads were saturated with fluorophores, there is no change in the ratio of fluorescent intensity between Beads 2 and 5 along the length of the tube because the beads are mixed homogeneously. However, when carrying out the third aspect of the invention, the flow rate of sample, and the binding affinity of constituents will be selected, so that intensity drop off does occur. That will enable the method to be used to quantify the analyte(s).

## EXAMPLE 3 – PREPARATION OF COLUMNS

Glass tubes, approximately 1.2 mm in diameter, were made from conventional Pasteur pipettes by cutting the pipette above the flute. This allows for rapid and easy filling of the narrower part of the tube. The dry tube was packed with a light cotton wool plug created by pushing loose cotton wool into the tube. The final length of the plug was 1-2 mm. The column was filled with phosphate buffered saline (PBS) buffer, 140mM NaCl and 10mM Sodium phosphate, pH 7.4 and observed for flow. Tubes with overly compressed plugs were rejected.

The tubes were then fed with a small amount of Sephadex G-50 beads swollen in buffer. These beads had an average diameter of 50 µm. The Sephadex bead bed was 2-3 mm in depth. Onto this bed an aliquot of Bangs Labs diamagnetic beads (8.03 µm diameter) derivatized with streptavidin on their surfaces was added. The diamagnetic beads occupy a depth of 6-8 mm and are drawn down into the tube using an external magnet. The diamagnetic beads were covered with a further 2-3 mm of Sephadex G-50 beads to produce the column. The Sephadex beads provide a non-fluorescent neutral support from which to establish the baseline for material adsorbed to the streptavidin-coated beads. Solutions run through the bed by gravity.

## EXAMPLE 4 – SAMPLE MEASUREMENT IN A TUBE

Biotin-4-fluorescein, which binds to streptavidin through the biotin linkage, was used as an analyte to detect in columns made according to Example 3. 150 μM of biotin-4-fluorescein was used. Aliquots of 0, 0.25, 0.5, 0.75, 1, 2, 3 and 10 μL of stock biotin-4-fluorescein solution were made up to 10 μL and each sample was added to a separate column. The solution flowed into the column at approximately 10 μL/3 hours.

Columns were then washed for several hours at room temperature (usually overnight) with fresh buffer. At the completion of each run, the flute section of each of the tubes was broken off, and the 1.2 mm tube of glass containing the column was mounted on a glass microscope slide. This slide was then inverted on the motorized stage of an Olympus inverted fluorescence microscope. The stage is manufactured by Mad City Labs and can move in X-Y directions with better than 1 μm accuracy. The Olympus objective is x20 oil immersion. The motorized stage allows accurate positioning along the axis of the tube. The fluorescence profile of analyte along the axis of the tube can readily be ascertained by measuring the peak height of the signal at any one station.

The excitation wavelength was 473 nm, and measurements were taken at 0.5 mm intervals along the tube. The signal generally occurs at a longer wavelength (higher nm) and is dye dependent.

Reproducibility of signal was established by moving the tube several millimetres axially and then returning the tube to its original position. This is shown in Figures 28 to 35.

Intensity was measured at six intervals for the tube containing 0.25 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 13a to 13f. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 20.

Intensity was measured at five intervals for the tube containing 0.5 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 14a to 14e. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 21.

Intensity was measured at seven intervals for the tube containing 0.75 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 15a to 15g. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown

in Figure 22.

Intensity was measured at eight intervals for the tube containing 1 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 16a to 16h The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 23.

Intensity was measured at eleven intervals for the tube containing 2 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 17a to 17k. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 24.

Intensity was measured at sixteen intervals for the tube containing 3 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 18a to 18p. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 25.

Intensity was measured at sixteen intervals for the tube containing 10 μL of stock biotin-4-fluorescein solution. The fluorescent measurement is shown in Figures 19a to 19p. The intensity of the relevant wavelength (508.4 nm) for each interval was plotted position along tube against fluorescence, as shown in Figure 26.

The area under each curve was calculated and plotted as shown in Figure 27. This plot shows that the peak height of the fluorescence signal is linearly related to concentration.

The preferred embodiment system will be programmed to extract the data and perform the necessary calculations to determine the quantity, and preferably concentration, of the analyte. The reader is referred to the section entitled "Quantification of analyte in sample using radiation intensity data".

## EXAMPLE 5

The columns prepared according to example 3 can first be spiked with 1 μL of a biotin-phycoerythrin (PE) solution. This excites at the same wavelength as biotin-4-fluorescein (488 nm laser line), but emits at a longer wavelength. The biotin-PE will bind to the beads at the top of the column and demonstrate

a spike of fluorescence as the column axis moves progressively across the objective. A biotin-4-fluorescein solution can then be flowed into the tube. This solution will bind to spare streptavidin molecules, establishing a fluorescent profile for biotin-4-fluorescein. This experiment demonstrates the use of an analyte spike of one colour to calibrate a column for a second analyte of unknown concentration.

The preferred embodiment system will be programmed to extract the data and perform the necessary calculations to determine the quantity, and preferably concentration, of the analyte. The reader is referred to the section entitled "Quantification of analyte in sample using radiation intensity data".

## EXAMPLE 6 – PREPARATION OF TUBES

Bangs Labs diagmagnetic beads (8.03 μm diameter) derivatised with streptavidin were mixed with DSB-X biotinylated donkey anti-goat IgG (2mg/mL Invitrogen D20698) at saturating concentrations at room temperature overnight. The antibody coated beads were then loaded into columns as described in Example 3 above.

Five antibodies produced in goat (either anti mouse or anti rabbit) were run through these columns. Each antibody was coupled to a different Alexa dye. The dye-antibody combination is shown below:

| | | |
|---|---|---|
| Alexa 546 – goat anti-mouse IgG (H & L) | 2 mg/mL | Invitrogen A11003 |
| Alexa 555 – goat anti-mouse IgG (H & L) | 2 mg/mL | Invitrogen A21422 |
| Alexa 568 – goat anti-mouse IgG (H & L) | 2 mg/mL | Invitrogen A11004 |
| Alexa 594 – goat anti-mouse IgG (H & L) | 2 mg/mL | Invitrogen A11005 |
| Alexa 532 – goat anti-rabbit IgG (H & L) | 2 mg/mL | Invitrogen A11009 |

The fluorescent emission wavelength peaks of the dyes used were 532, 546, 555, 568 and 594 nm. These were used at high concentrations that were to saturate the column (concentration 2mg/mL).

The anti-goat antibodies on the column bind only antibodies produced in goat. Other proteins or antibodies produced in other animals will not remain on the column.

Two columns were set up for the experiment. The first column had saturating concentrations of all five goat antibodies run through it (referred to as Tube 3). Tube 4 had saturating concentrations of goat antibodies labelled with Alexa dye 555 and 594 run through it.

Each tube was prepared for fluorescence microscopy as in Example 4. The same fluorescence microscope was used. The excitation wavelength was 532 nm. Measurements were taken at 0.5 mm intervals along the tube. The light emitted was analysed using the apparatus shown in figures 1 and 12 to provide intensities at the wavelengths emitted (with a narrow block on light of the excitation wavelength, 532 nm).

## EXAMPLE 6 - DATA ANALYSIS

The tubes were analysed using the system of Figures 1 and 12.

Silicon photo detectors including silicon CCD arrays such as the Princeton Instruments (PI) wide aspect spectroscopic TEC cooled camera have a pixel element signal response proportional to the intensity of the incident light provided the device is out of saturation. The height of the spectroscopic profile is therefore proportional to the light intensity on the photo detector element at the reported wavelength. Here arbitrary units are used as the height of the spectroscopic trace varies linearly with the total integration time and therefore total energy dose per detection element.

Spectral information was decomposed using the following method: Base or basis dyes under cover slips are individually examined for their characteristic spectra under the laser excitation wavelength which is to be used for the remaining experiment. Here the laser excitation wavelength was the green colour 532nm. The five individual base spectra for Alex Fluor dyes 532 (line 1 in Figures 28 and 29), 546 (line 2 in Figures 28 and 29), 555 (line 3 in Figures 28 and 29), 568 (line 4 in Figures 28 and 29), and 594 (line 5 in Figures 28 and 29) were recorded and the trace height normalised. The laser excitation wavelength, 532 nm, was blocked by the DMD. As the width of the 532 nm laser excitation line was physically narrower than the 532 nm fluorescence emission spectrum width, sufficient data relating to the 532 nm fluorescence spectrum was delivered to the Detector to analyse the fluorescence, despite the laser excitation line being blocked by the DMD. These data were then assigned within the MATLAB decomposition software as the basis spectral profiles with which to decompose mixed spectra arising from combinations of these five dyes. In addition data was acquired in the absence of

any fluorescent dye allowing baseline data files to be obtained for the residual excitation light emanating from the image of the laser line on the DMD as well as scattered excitation light falling onto the active (directed towards the PI camera) region of the DMD. These final two data files are assigned within the decomposition software as noise sources contributing to the final mixed spectra from the various dye combinations.

Spectroscopic data was recorded as a function of longitudinal position within tubes 3 and 4 (see experimental data). Spectra were recorded at every 500 micrometers for a total traversal distance of 0.00-7.50 mm for tube 3 (16 data collection points) 0.00-9.00 mm for tube 4 (19 data collection points). Data was exported from the PI software 'Winspec' as an 8bit tif image which was then loaded within the decomposition software running under MATLAB. The decomposition software reported the contributions from each dye through a process of fitting linear summations of the individual basis spectra to the combined spectra. The sum of the contributions adds to 100%. Noise contributions are removed and the signals for the dyes were renormalised such that their sum yields 100%.

The fluorescent intensities for tube 3 showed binding of all five fluorescently labelled antibodies. Both the antibodies used in tube 4 also showed binding. The data in Figures 28 and 29 show the percentage intensity from each fluorescent antibody. The amount of binding was relatively constant over most of the 9 mm length examined but became variable at the end due to problems of column uniformity.

This experiment shows the ability of the apparatus of the invention to determine levels of the five fluorescent antibodies along a column.

Preferred embodiments of the invention have been described by way of example only and modifications may be made thereto without departing from the scope of the invention.

For example, the system may comprise one or more suitable optical components between any of the other components mentioned above. For example, the system may have one or more reflective and/or one or more refractive elements between any of the other components mentioned above. Additionally, one or more optical fibres may be used between components to enable the components to be spaced remotely from one another.

Rather than using a diffraction grating or prism, a concave holographic grating could receive the returned radiation and disperse and focus that onto the SMD. A further concave holographic grating

configured to operate in reverse, to focus the dispersed spectrum from the SMD onto the detector. A small photodetector or point detector is particularly suited to a system having a concave holographic grating that focuses the returned radiation to be analysed from the SMD onto the detector.

Other alternatives are listed in the "Summary of Invention" section.

## Industrial Application

It will be appreciated by those skilled in the art that the systems and methods of the present invention have many applications including genetic testing; detection, diagnosis and prognosis of medical conditions or diseases including cancer, analysis of embryos particularly single cell analysis of pre-implantation human embryos at a very early stage for in vitro fertilisation. The systems and methods of the present invention will be particularly advantageous where the sample size or analyte concentration is very low.

## CLAIMS

1. An analysis system comprising:

a sample region for receipt of a sample to be analysed;

at least one source of excitation radiation; and

a wavelength-inspecific beam splitter that operatively couples the source of excitation radiation with the sample region to deliver some of the excitation radiation from the source of excitation radiation to a sample to be analysed in the sample region, the beam splitter configured to receive returned radiation from a sample to be analysed in the sample region and to transmit at least some of the returned radiation, wherein the returned radiation comprises residual excitation radiation and radiation to be analysed;

a dispersion element configured to disperse the returned radiation from the sample to be analysed that has been transmitted by the beam splitter;

a selectively switchable micromirror device configured to receive returned radiation that has been dispersed by the dispersion element; and

a detector to detect radiation that is received from the switchable micromirror device;

wherein the switchable micromirror device is configured to remove at least a major part of the residual excitation radiation from the returned radiation that is passed to the detector.

2. An analysis system as claimed in claim 1, wherein the source(s) of excitation radiation is/are a laser source.

3. An analysis system as claimed in claim 2, comprising a plurality of sources of excitation radiation, wherein the sources of excitation radiation comprise a plurality of laser sources, each of which is configured to provide an excitation radiation with a different wavelength or a different wavelength band.

4. An analysis system as claimed in any one of claims 1 to 3, wherein the beam splitter is configured to reflect excitation radiation to the sample region.

5. An analysis system as claimed in claim 4, wherein the beam splitter is configured to reflect only a small proportion of the excitation radiation to the sample region, and allows the majority remainder of the excitation radiation to pass through the beam splitter.

6. An analysis system as claimed in claim 5, wherein a surface of the beam splitter is configured to reflect between about 5% and about 15% of the excitation radiation to the sample region.

7. An analysis system as claimed in claim 6, wherein the surface of the beam splitter is configured to reflect about 10% of the incoming excitation radiation to the sample region.

8. An analysis system as claimed in claim 6 or 7, wherein at least a major part of the returned radiation from the sample to be analysed that is delivered to the beam splitter, passes through the surface of the beam splitter.

9. An analysis system as claimed in claim 8, wherein about 85 to about 95% of the returned radiation that is delivered to the beam splitter, passes through the surface of the beam splitter.

10. An analysis system as claimed in claim 9, wherein about 90% of the returned radiation that is delivered to the beam splitter, passes through the surface of the beam splitter.

11. An analysis system as claimed in any one of claims 6 to 10, wherein the surface of the beam splitter that reflects the excitation radiation to the sample region is a front surface of the beam splitter, and the beam splitter also has a rear surface that reflects and transmits in similar proportions to the front surface.

12. An analysis system as claimed in claim 11, wherein the beam splitter is configured so that excitation radiation being reflected by the rear surface of the beam splitter toward the sample region does not significantly interfere with the excitation radiation being reflected by the front surface of the beam splitter toward the sample region.

13. An analysis system as claimed in claim 12, wherein the beam splitter has a thickness of at least about 2 mm.

14. An analysis system as claimed in claim 13, wherein the beam splitter has a thickness of about 10 mm.

15. An analysis system as claimed in claim 14, wherein the beam splitter is sufficiently thin that the excitation radiation being reflected by the front and rear surface is directed to the sample in the sample region.

16. An analysis system as claimed in claim 15, wherein the beam splitter has a thickness of between about 60 and about 150 micrometers.

17. An analysis system as claimed in claim 16, wherein the beam splitter has a thickness of between about 80 and about 100 micrometers.

18. An analysis system as claimed in any one of claims 1 to 17, wherein the beam splitter is made of a material which offers substantially linear optical transmission over a range of at least about 350 nm to about 800 nm.

19. An analysis system as claimed in claim 18, wherein the material is a glass or plastic material.

20. An analysis system as claimed in any one of claims 1 to 19, wherein the dispersion element comprises a concave holographic grating.

21. An analysis system as claimed in any one of claims 1 to 20, wherein the SMD has a plurality of columns of micromirrors and a plurality of rows of micromirrors, and is arranged so that either each column or each row of micromirrors, or a plurality of adjacent columns or rows of micromirrors, corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element.

22. An analysis system as claimed in claim 21 or claim 22, wherein the SMD is arranged so that each column, or plurality of adjacent columns, of micromirrors corresponds to a respective wavelength or wavelength range of the returned radiation from the dispersion element, and at least a major part of the residual excitation radiation can be removed from the radiation passed to the detector by turning one or more columns of the SMD corresponding to the residual excitation radiation line.

23. An analysis system as claimed in claim 21 or claim 22, wherein the SMD is controlled so that the radiation to be analysed is passed to the detector a single column at a time, or a plurality of adjacent columns at a time, so that radiation corresponding to a single wavelength or wavelength range is passed to the detector at a time.

24. An analysis system as claimed in claim 21, wherein the SMD is controlled to remove only the residual excitation radiation, and to direct all of the radiation to be analysed to the detector at once.

25. An analysis system as claimed in any one of claims 1 to 24, comprising a plurality of sources of excitation radiation, wherein the excitation radiation from each of the sources is delivered to the sample region by the wavelength-inspecific beam splitter.

26. An analysis system as claimed in any one of claims 1 to 25, wherein the source(s) of excitation radiation is/are configured to supply excitation radiation at wavelength(s) within the range of about 400 nm to about 1400 nm.

27. An analysis system as claimed in claim 26, wherein the source(s) of excitation radiation is/are configured to supply excitation radiation at wavelength(s) in the range of about 400 nm to about 800 nm.

28. An analysis system as claimed in any one of claims 1 to 27, wherein the system is configured to determine from the radiation to be analysed that is received by the detector, analysis data relating to the received radiation.

29. An analysis system as claimed in claim 28, wherein the system is configured to determine from the radiation received by the detector, the quantity of one or more analytes in a sample.

30. An analysis system as claimed in claim 29, wherein the system is configured to quantify at least five analytes in a sample from a single source of excitation radiation.

31. An analysis system as claimed in claim 30, wherein the system is configured to quantify twenty analytes in a sample, from four sources of excitation radiation.

32. An analysis system as claimed in any one of claims 28 to 31, wherein the system comprises a processing unit that is programmed to determine the quantity of one or more analytes.

33. An analysis system as claimed in any one of claims 28 to 32, wherein the system is configured to provide a quantification measurement of an analyte in the sample, based on total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, for a plurality of points in the sample region.

34. An analysis system as claimed in any one of claims 1 to 33, wherein the system is configured to provide a quantification measurement of an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, corresponding to the analyte, to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

35. An analysis system as claimed in any one of claims 1 to 34, wherein the system is configured to provide a quantification measurement of an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range, corresponding to the analyte to programmed factor(s) relating to the analyte.

36. A method of analysing a sample, comprising:

providing an analysis system as claimed in any one of claims 1 to 35;

positioning the sample in the sample region;

delivering excitation radiation to the sample via the wavelength-inspecific beam splitter to generate returned radiation comprising residual excitation radiation and radiation to be analysed;

receiving dispersed returned radiation from the dispersion element on the switchable micromirror device; and

operating the switchable micromirror device to remove at least a major part of the residual excitation radiation from the returned radiation that is passed to the detector but to deliver at least some of the radiation to be analysed to the detector.

37. A method as claimed in claim 36, wherein the sample contains one or more target analyte(s), and the method comprises quantifying the target analyte(s) in the sample.

38. A method as claimed in claim 37, wherein the sample contains five target analytes, and the method comprises quantifying the five target analytes.

39. A method as claimed in claim 38, wherein the sample contains twenty target analytes, and the method comprises quantifying the twenty target analytes.

40. A method as claimed in any one of claims 36 to 39, wherein the method comprises quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to the analyte, to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

41. A method of any one of claims 36 to 40, wherein the method comprises quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to programmed factor(s) relating to the analyte.

42. A method of quantifying at least one target analyte in a sample, comprising:

providing a sample region for receipt of a sample to be analysed, the sample region comprising a group of receptors along a length of the sample region that are directly or indirectly immobilised on a surface of the sample region;

providing an analysis system comprising a detector;

providing a sample to be analysed;

passing the sample along the length of the sample region comprising receptors, whereby a specific analyte in the sample, if any, binds to the receptors;

labelling the target analyte either before or after passing the sample along the length of the sample region comprising receptors;

receiving radiation from the sample region for each of a plurality of points

along at least part of the length of the sample region or different parts of the sample region and delivering at least some of the radiation to the detector; and

quantifying the target analyte in the sample, if any, from the radiation passed to the detector.

43. A method as claimed in claim 42, wherein the group of receptors comprises a plurality of types of receptor, each type of receptor being specific for a respective target analyte, and at least two analytes are quantified.

44. A method as claimed in claim 42 or 43, wherein the group of receptors comprises at least three types of receptors specific for at least three target analytes, and at least three target analytes are quantified.

45. A method as claimed in any one of claims 42 to 44, wherein the group of receptors comprises at least five types of receptors specific for at least five target analytes, and at least five target analytes are quantified.

46. A method as claimed in any one of claims 43 to 45, wherein the group of receptors comprises at least twenty types of receptors specific for at least twenty target analytes, and at least twenty target analytes are quantified.

47. A method as claimed in any one of claims 43 to 46, wherein the types of receptors are mixed substantially homogenously along the length of the sample region.

48. A method as claimed in any one of claims 43 to 46, wherein the types of receptors have separate distribution zones for each type of receptor along the length of the sample region.

49. A method as claimed in any one of claims 42 to 48, wherein the sample region comprises a matrix with bound receptors.

50. A method as claimed in any one of claims 42 to 49, wherein the sample region comprises a matrix of beads.

51. A method as claimed in claim 50, wherein the beads are magnetic and are held in place by a magnet.

52. A method as claimed in any one of claims 42 to 50, wherein the sample region is in a column.

53. A method as claimed in any one of claims 49, 50 or 52, wherein the matrix is retained in place by a porous substrate such as cotton wool or nylon mesh.

54. A method as claimed in any one of claims 42 to 53, wherein the receptors comprise receptors selected from the group consisting of antibodies and nucleic acid probes.

55. A method as claimed in claim 54, wherein the nucleic acid probe is a cDNA probe.

56. A method as claimed in claim 54, wherein the receptors comprise at least one type of antibody.

57. A method of any one of claims 42 to 56, wherein the target analyte is a protein, peptide or nucleic acid.

58. A method as claimed in any one of claims 42 to 57, wherein the sample is passed along the length of the sample region in a stepped or indexed manner.

59. A method as claimed in any one of claims 42 to 58, wherein the amount of target analyte bound to the group of receptors generally reduces along at least part of the length of the sample region comprising receptors so that more target analyte is bound to the sample region where the sample was administered than at the end opposite to where the sample was administered due to the previous binding of the analyte to the receptors.

60. A method as claimed in any one of claims 42 to 59, wherein quantifying at least one target analyte in the sample is based on the total intensity of the radiation received by the detector at a wavelength or wavelength range, for a plurality of points along the sample region.

61. A method as claimed in any one of claims 42 to 60, wherein the method comprises quantifying a plurality of analytes in the sample, based on total intensity of the radiation received by the detector at a plurality of wavelengths or wavelength ranges, for a plurality of points along the sample region.

62. A method as claimed in any one of claims 42 to 61, wherein the at least one target analyte is quantified by measuring the total intensity of the radiation received by the detector at a plurality of wavelengths or wavelength ranges, for a plurality of points along the sample region and across the sample region.

63. A method as claimed in any one of claims 42 to 62, wherein the radiation received from the sample region is light or UV light and the radiation delivered is fluorescent light or UV light.

64. A method as claimed in any one of claims 42 to 63, wherein the method includes a pre-concentrating step, which concentrates target analytes within the sample before analysing such analytes.

65. A method as claimed in claim 64, wherein the pre-concentrating step uses an ion exchanger to bind analytes, which are subsequently released in a small volume.

66. A method as claimed in claim 64, wherein the ion exchanger includes a cleavable linker joining an ionic group to a solid support.

67. A method of any one of claims 42 to 66, wherein the method comprises quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to the total intensity of the radiation to be analysed received by the detector at a wavelength or wavelength range corresponding to an analyte of a known quantity.

68. A method of any one of claims 42 to 67, wherein the method comprises quantifying an analyte in the sample by comparing the total intensity of the radiation to be analysed received by the detector at a wavelength corresponding to the analyte to programmed factor(s) relating to the analyte.

69. A method as claimed in any one of claims 42 to 68, wherein two or more analytes selected from the group comprising protein(s), peptide(s), DNA, RNA, carbohydrate(s) and lipid(s)are quantified from a sample.

70. An apparatus configured to perform the method of any one of claims 42 to 68.

71. A method as claimed in any one of claims 42 to 68, wherein the method is carried out using a system of any one of claims 1 to 35.

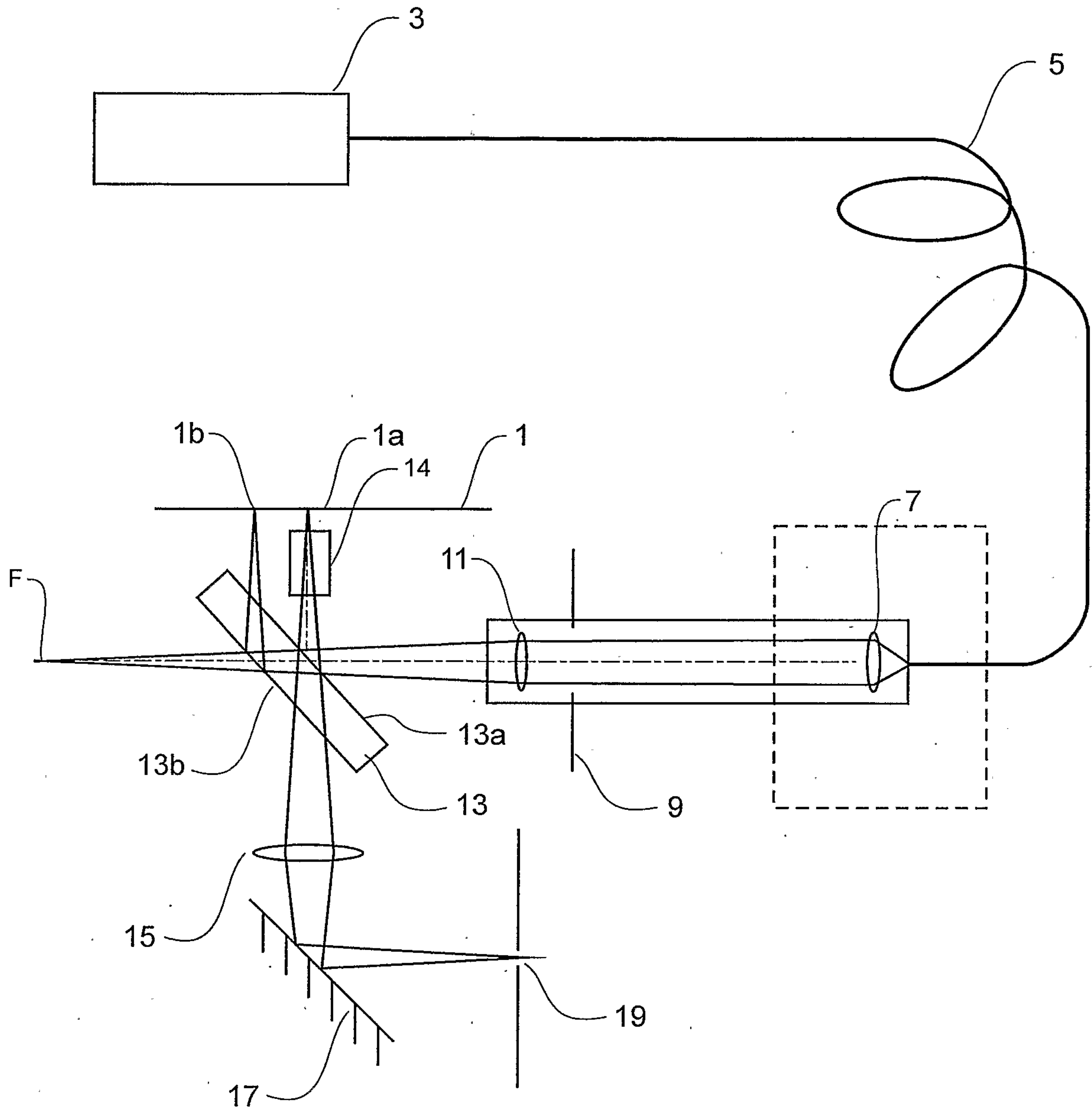
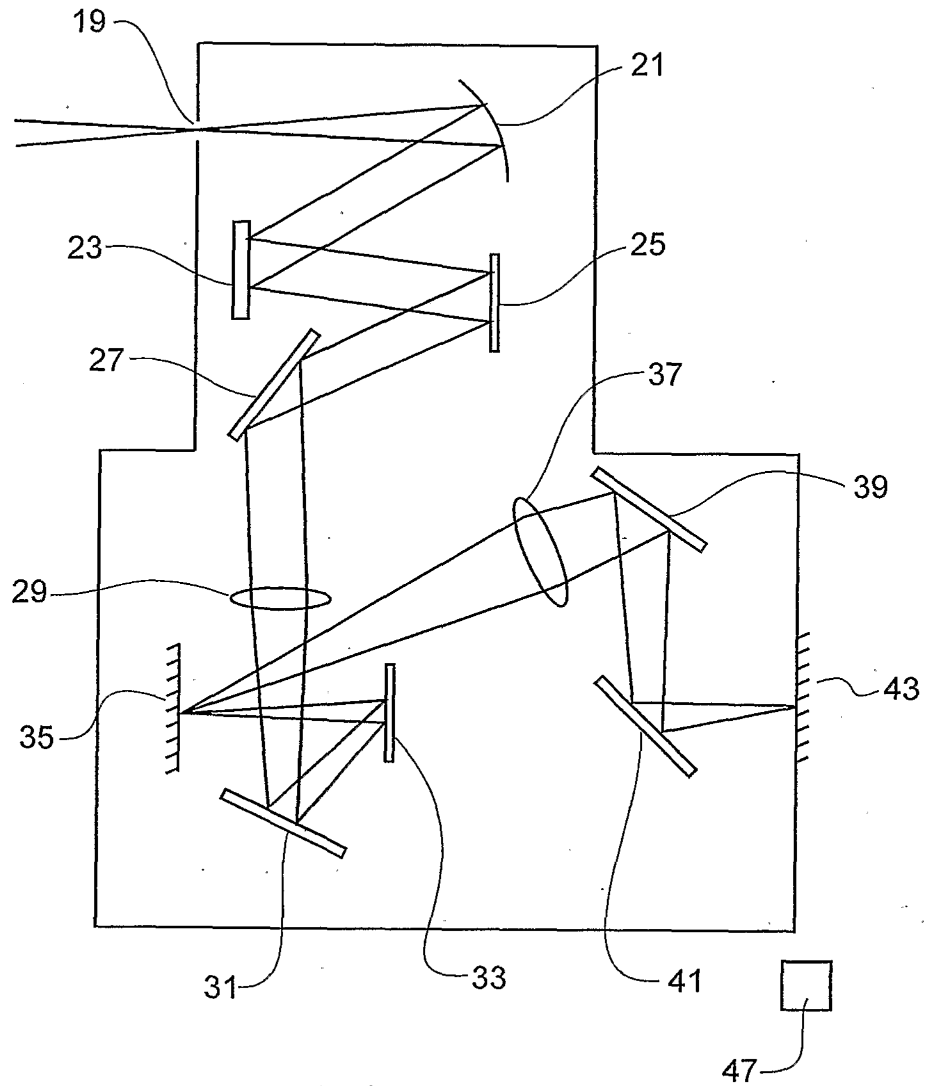



FIGURE 1




# FIGURE 2

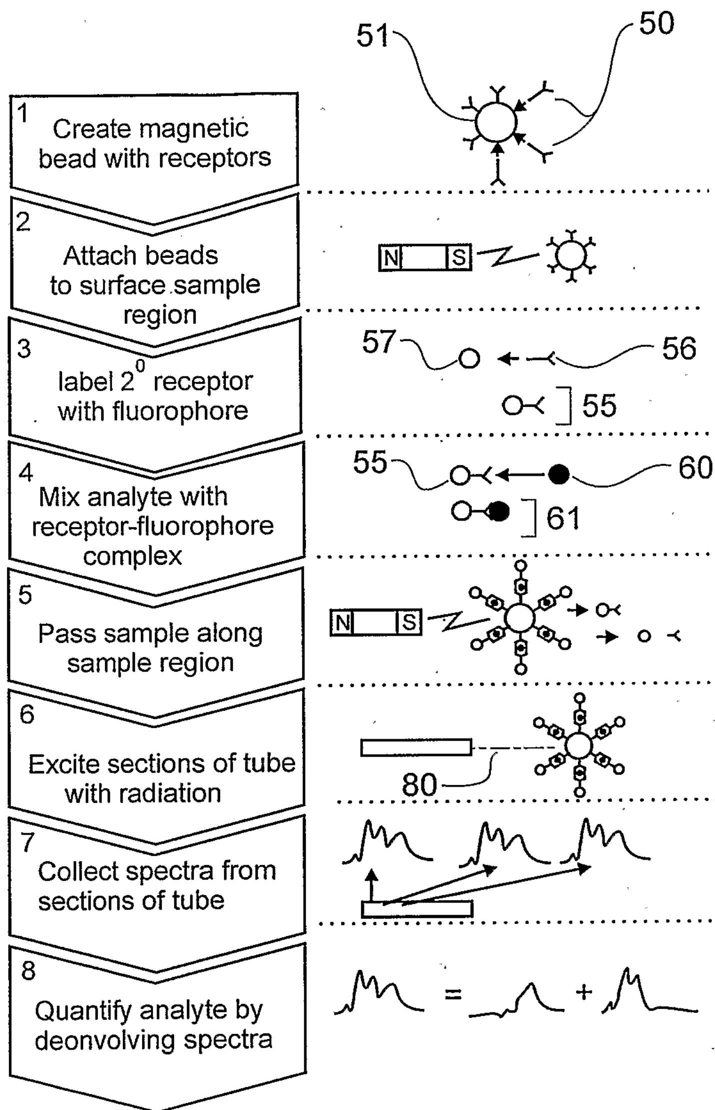



FIGURE 3

70a
first analyte
70b
second analyte
51
50a
50b
1
80
excitation radiation
returned radiation
81
57
55

**FIGURE 4**

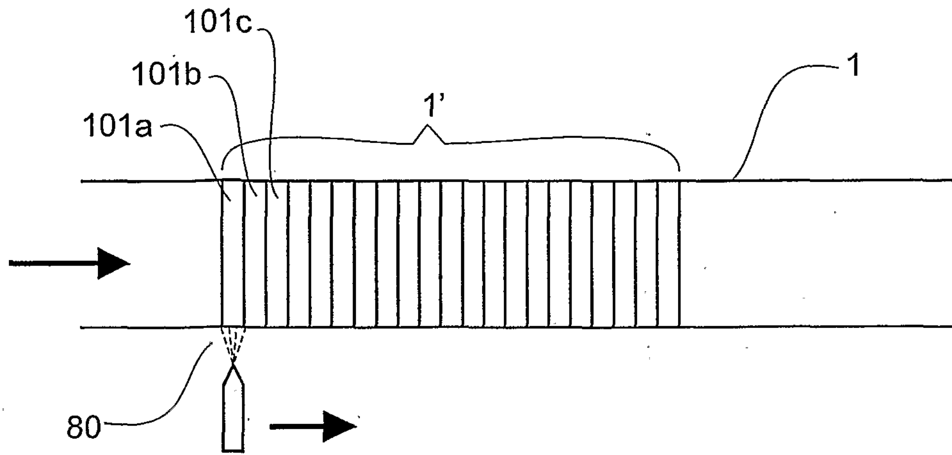



FIGURE 5

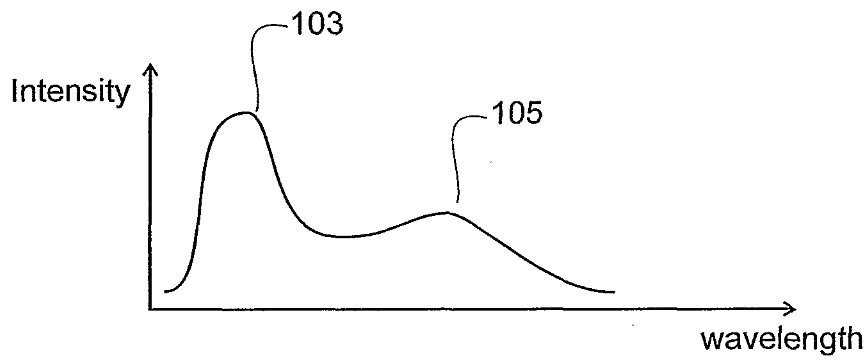



FIGURE 6a

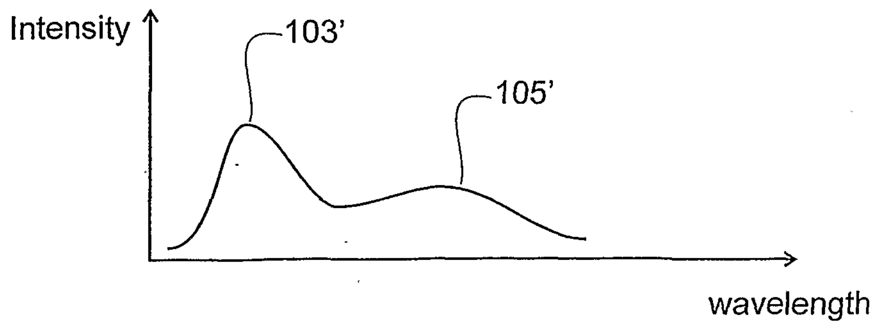



FIGURE 6b

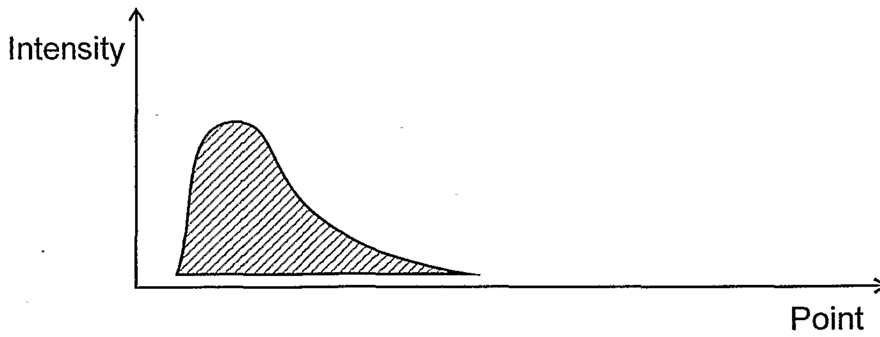



**FIGURE 7a**

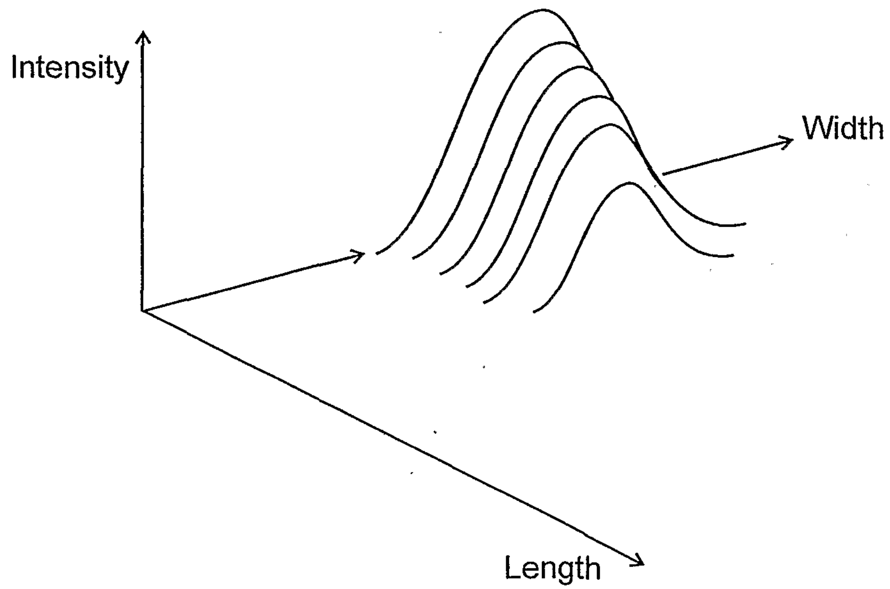



**FIGURE 7B**

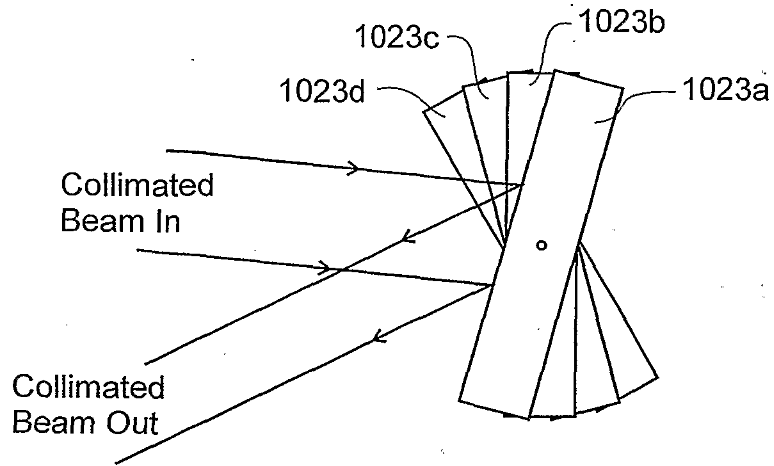



FIGURE 8a

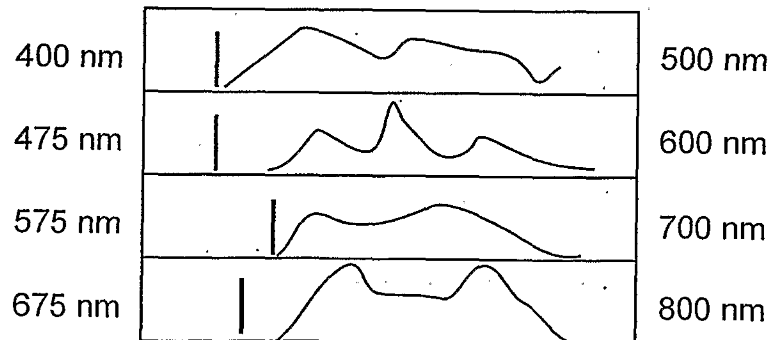



FIGURE 8b

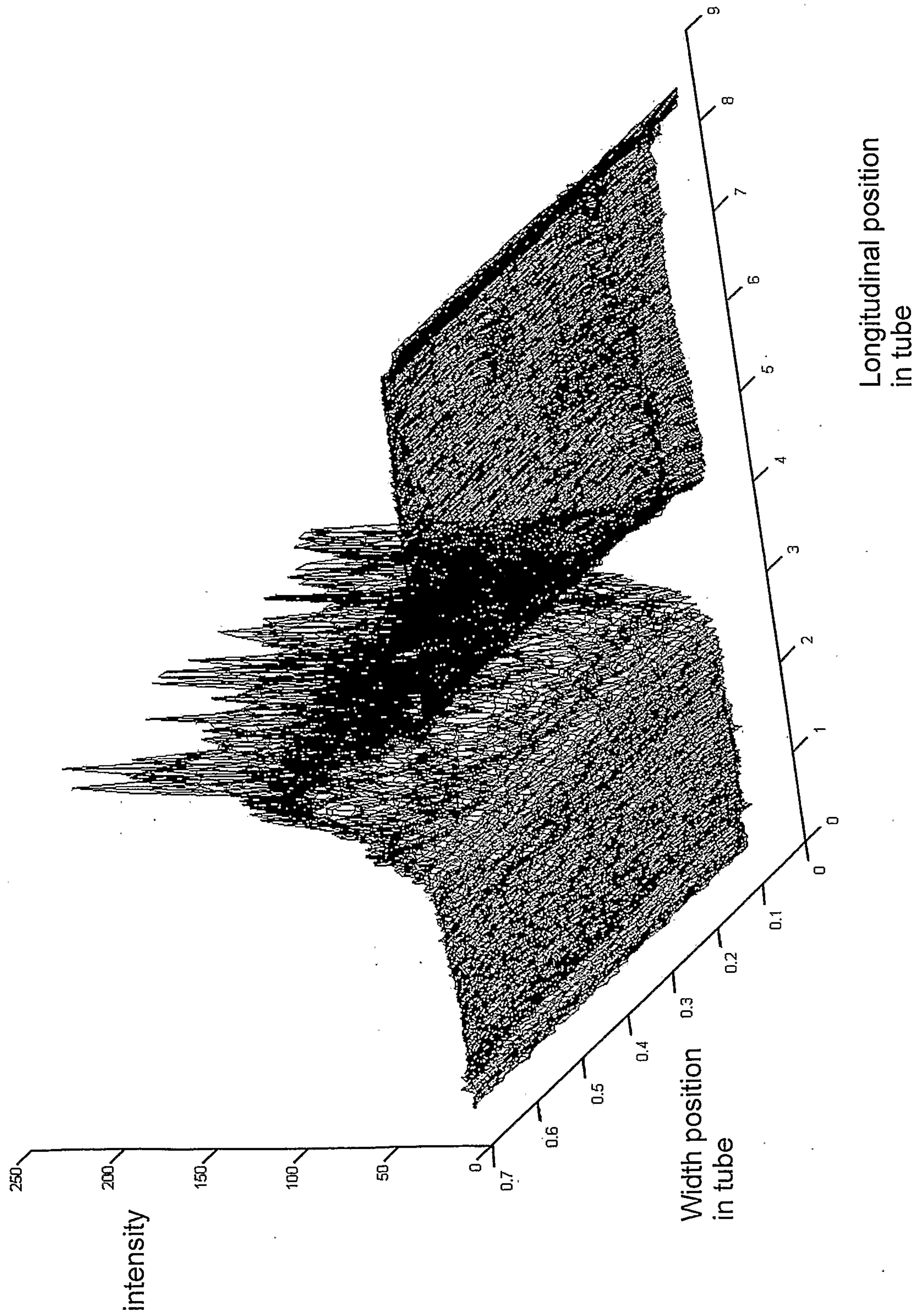



**FIGURE 9**

CCD: Unsaturated
16 bpp grayscale
1340 by 100 pixels
13-Dec-2007 12:10:42

CCD Horizontal axis (mm)

Binned Pixels

raw data

Convolved spectra taken at longitudinal position 500 um. Total length of bead filled tube ~1000um.

10.92 520.33 2386.59

12.64 569.19 7113.47

raw data

Area sum: 631462 (nm x Intensity)
Area 488: 151031
Area 514: 54845
Area 532: 0
Area 546: 0
Area 555: 274998

Sum of spectral contributions
Bead 5 spectral contribution
Bead 2 spectral contribution
Excitation light contribution (473nm)

deconvoluted

Wavelength (nm)

Stray excitation light (473 nm)

Bead 2

Not present

Not present

Bead 5

Alexa Fluor Dye

Contribution by each component

# FIGURE 10

Normalised sum of signals from beads 2 and 5 after the excitation light contribution has been subtracted

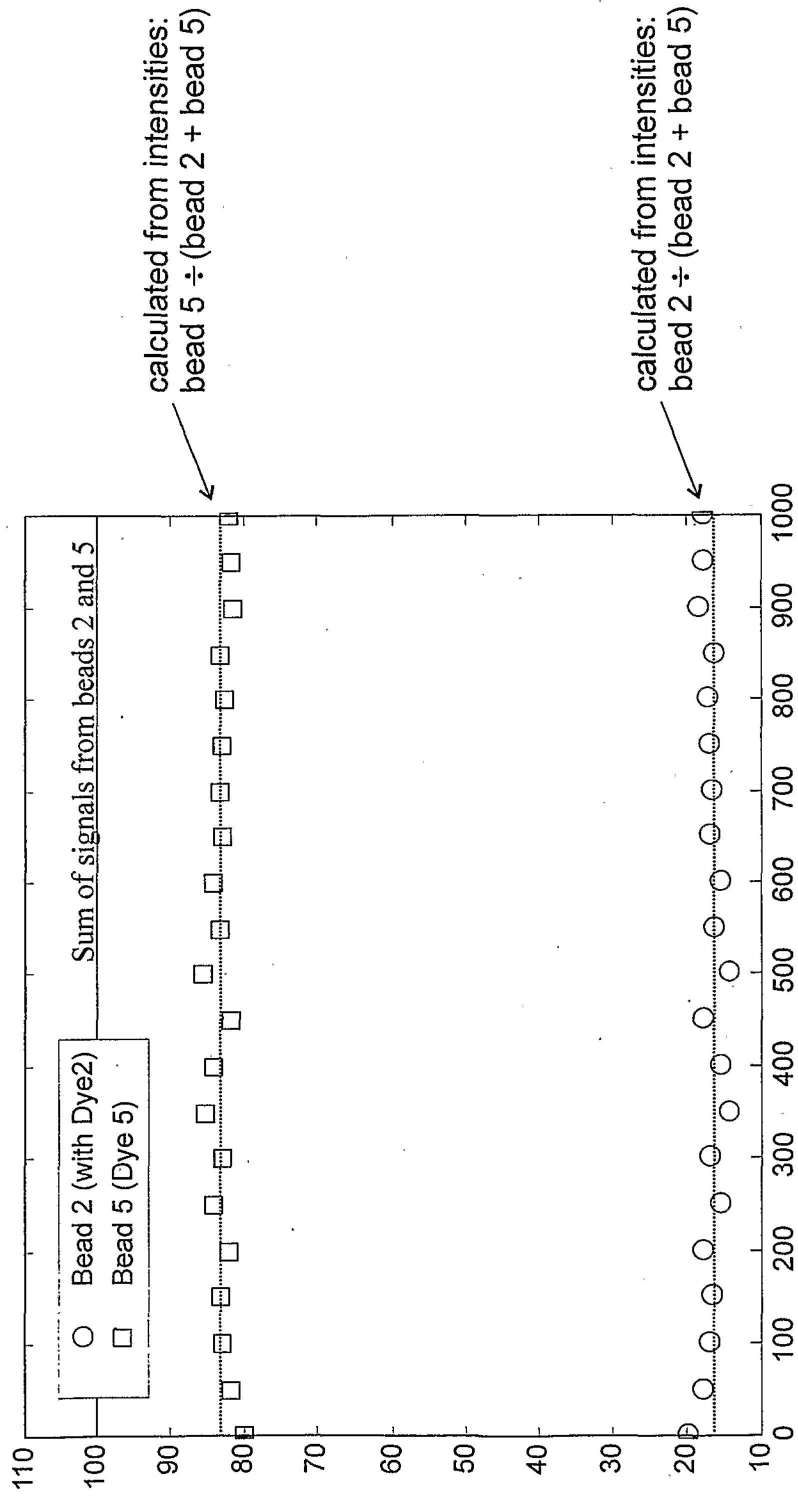
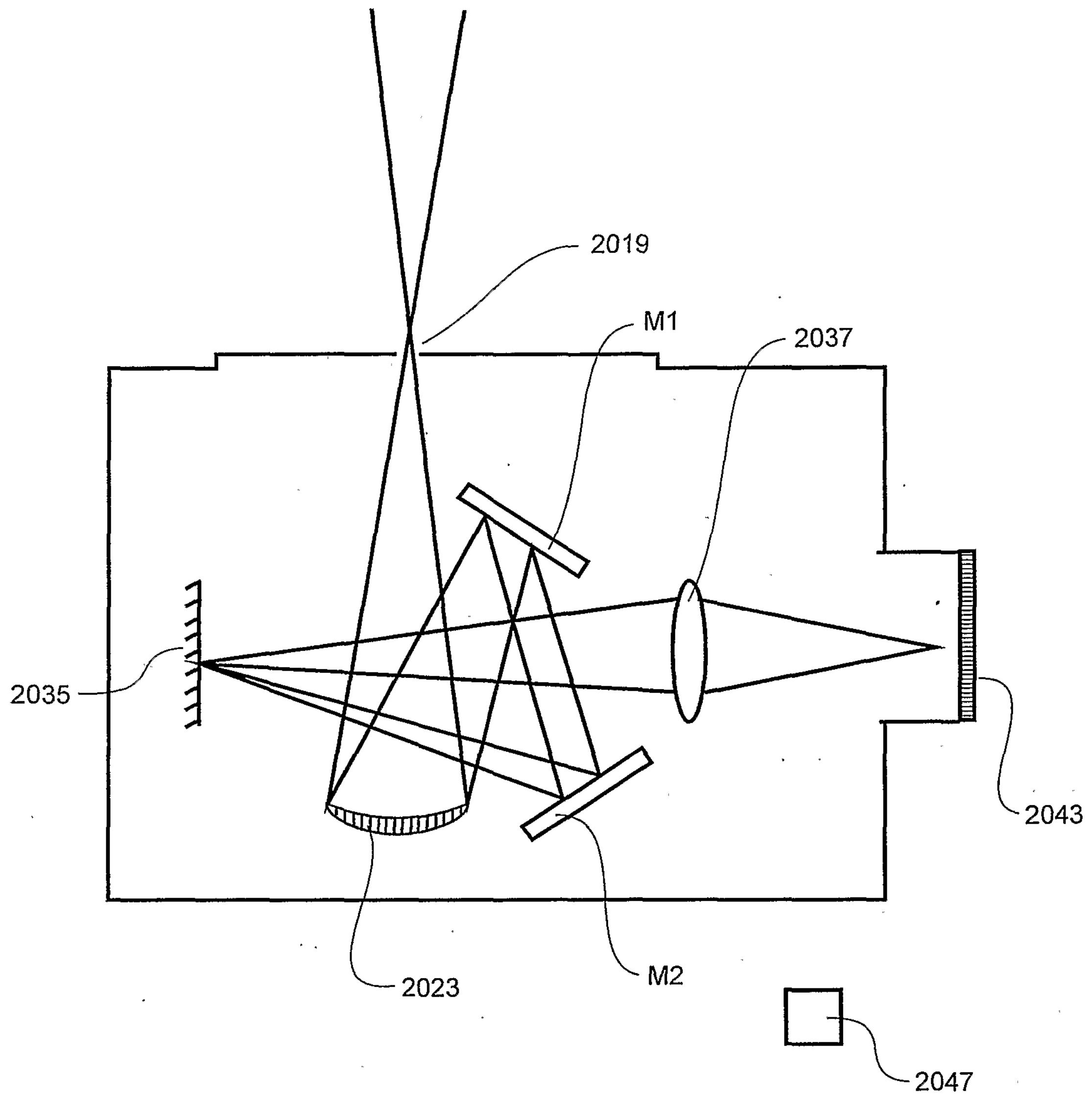



**FIGURE 11**




# FIGURE 12

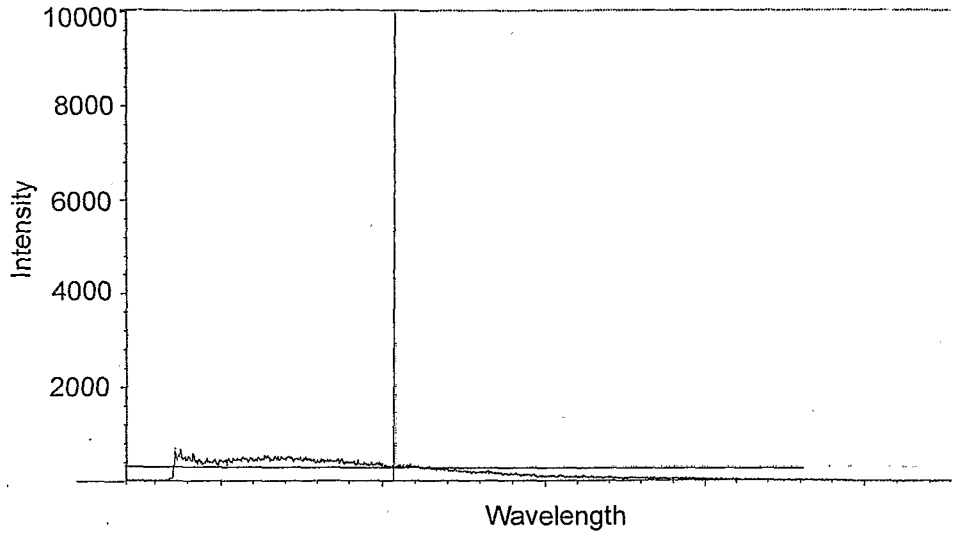



FIGURE 13a

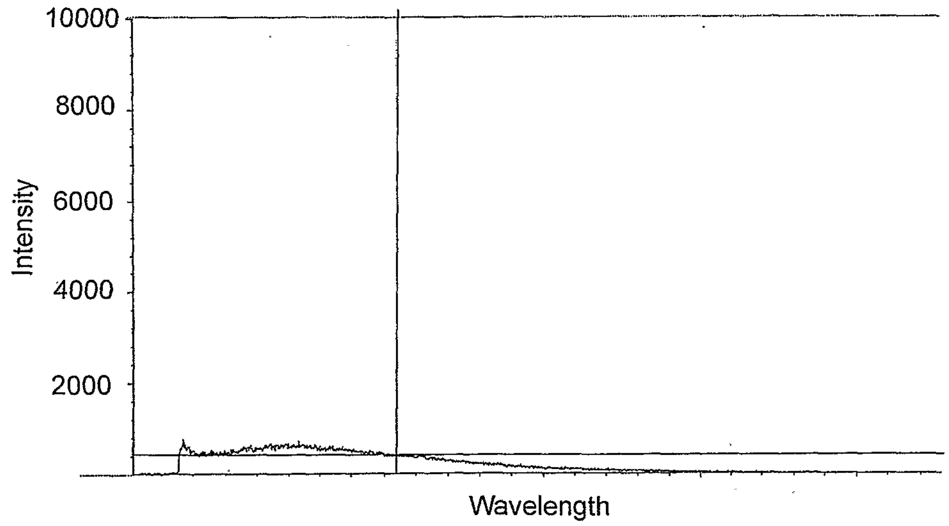



FIGURE 13b

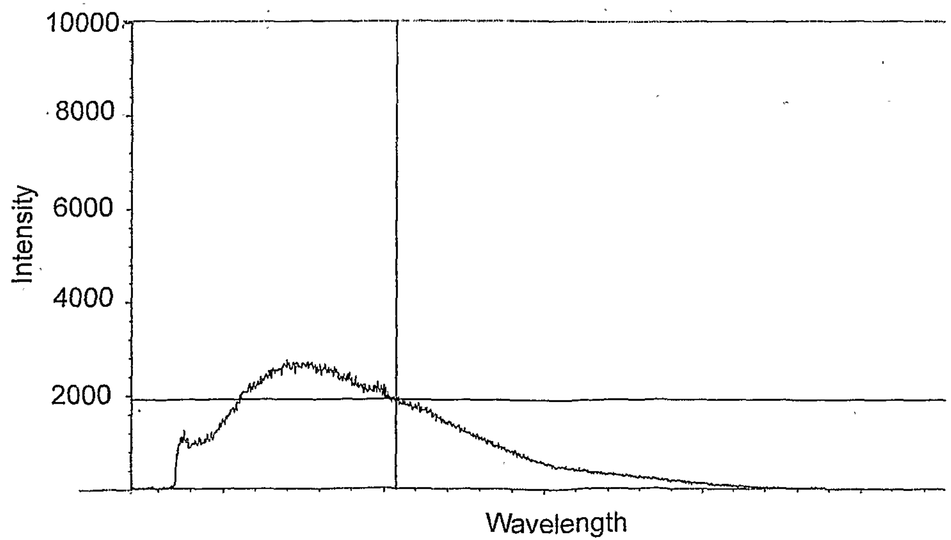



FIGURE 13c

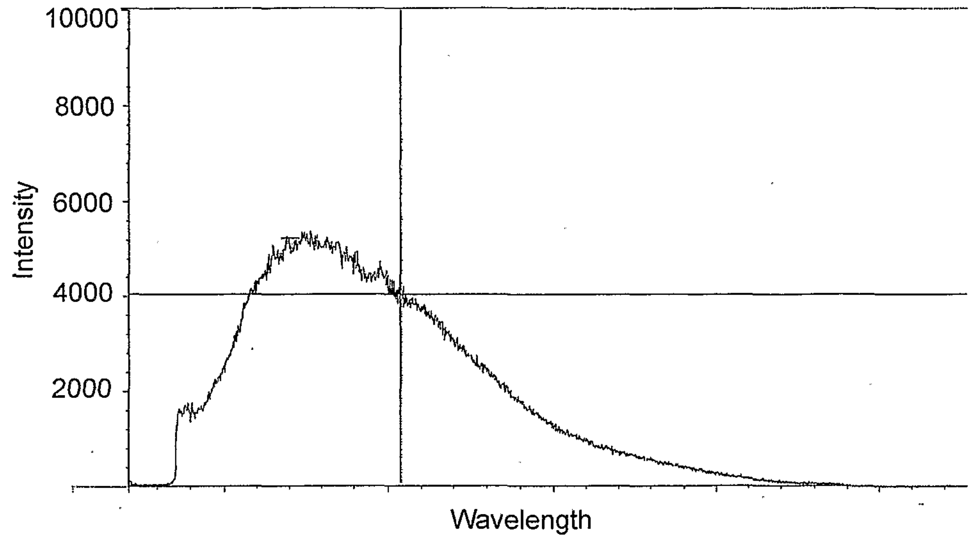



FIGURE 13d

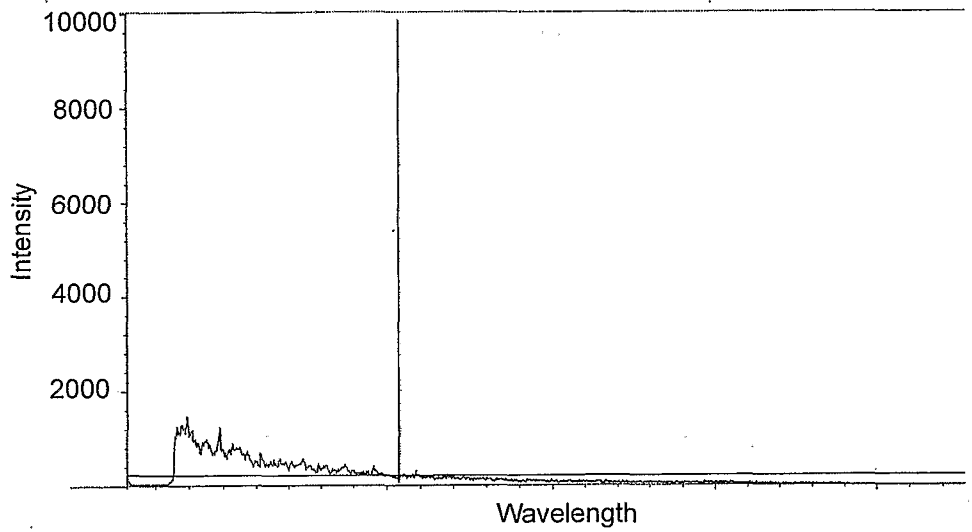



FIGURE 13e

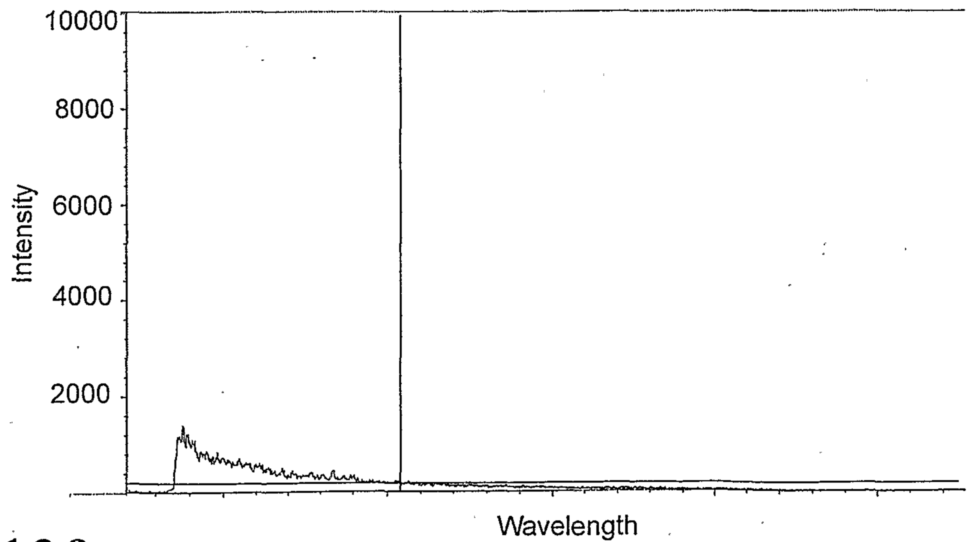
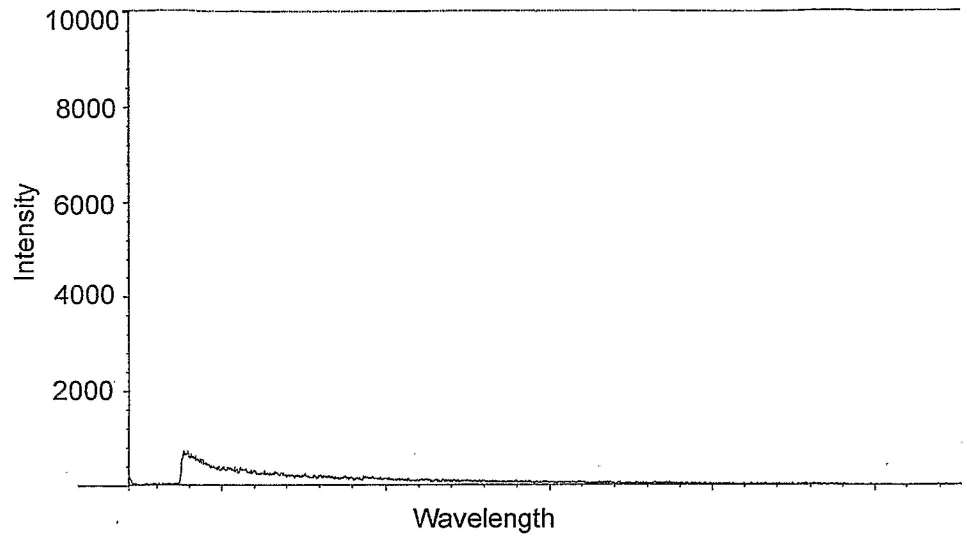
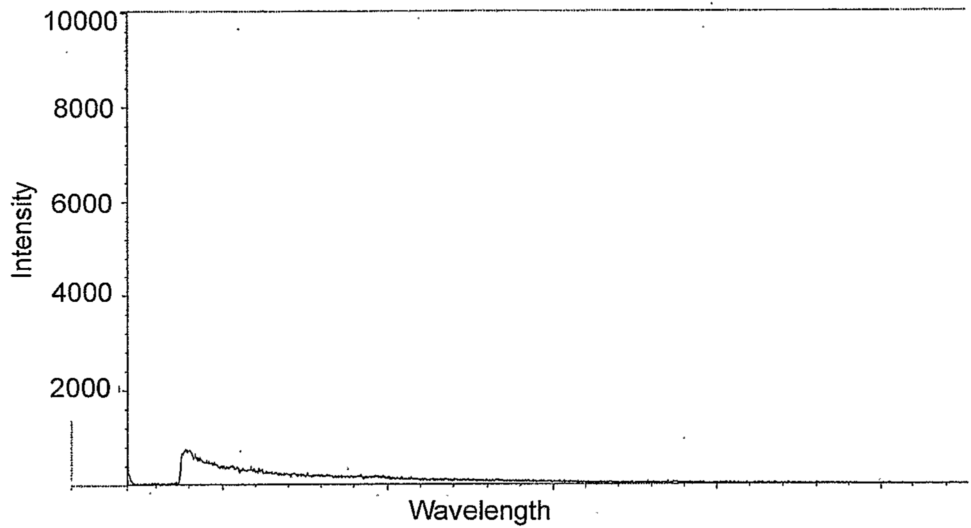


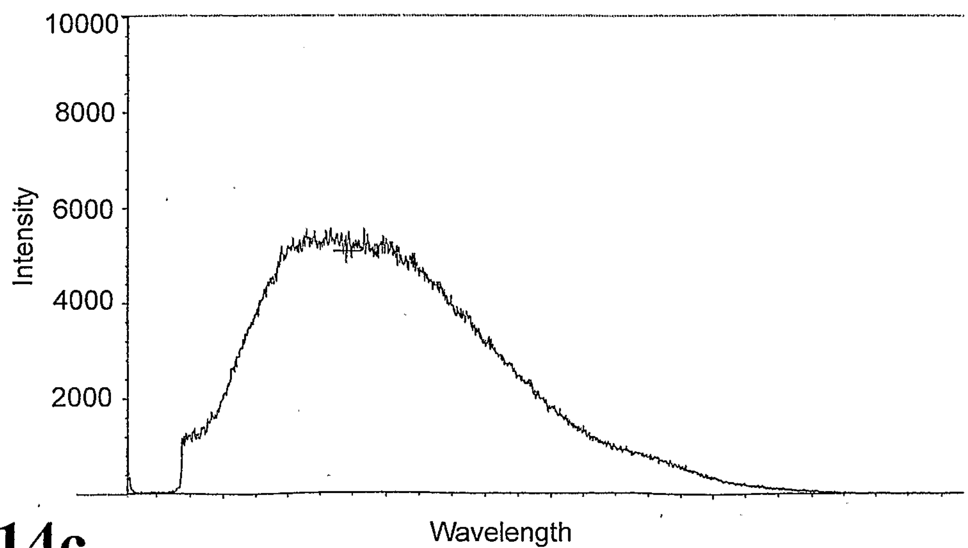

FIGURE 13f




# FIGURE 14a




# FIGURE 14b




# FIGURE 14c

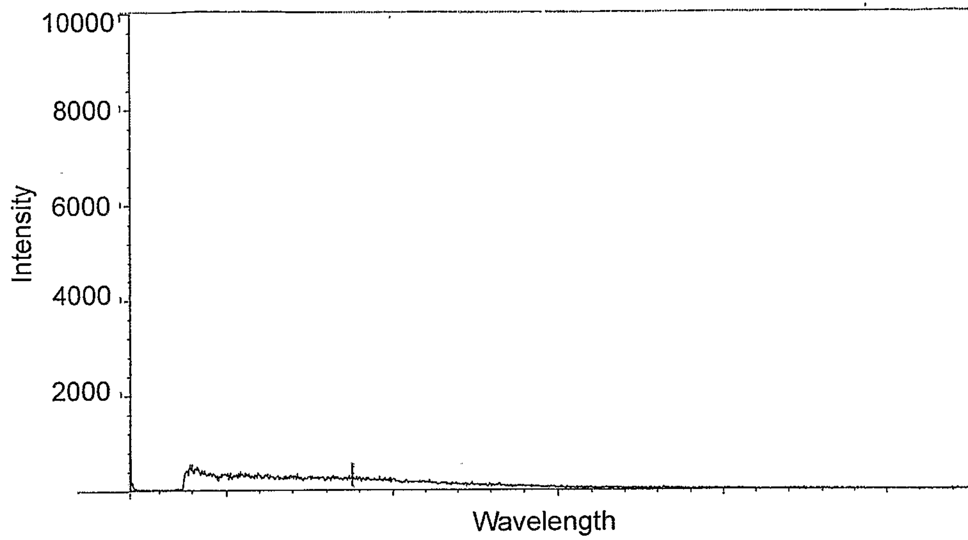



FIGURE 14d

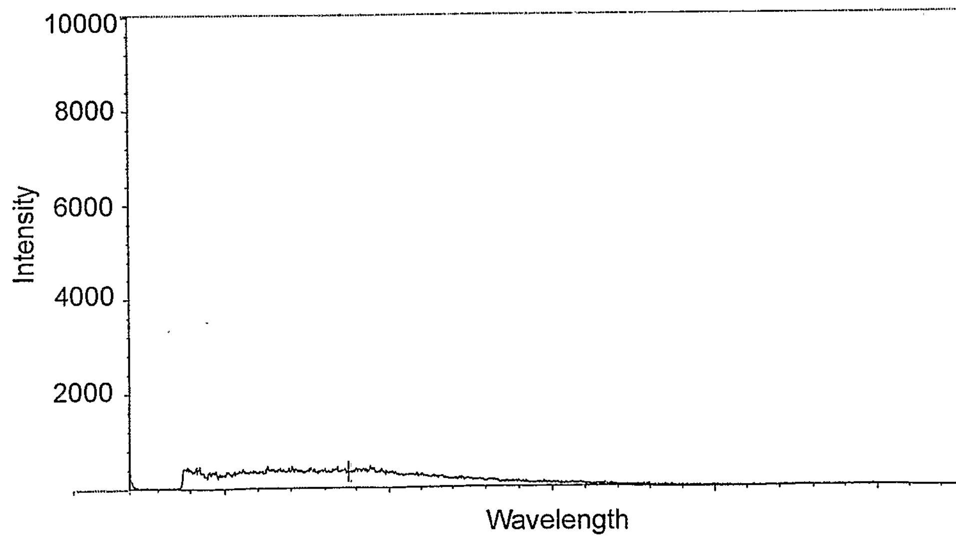



FIGURE 14e

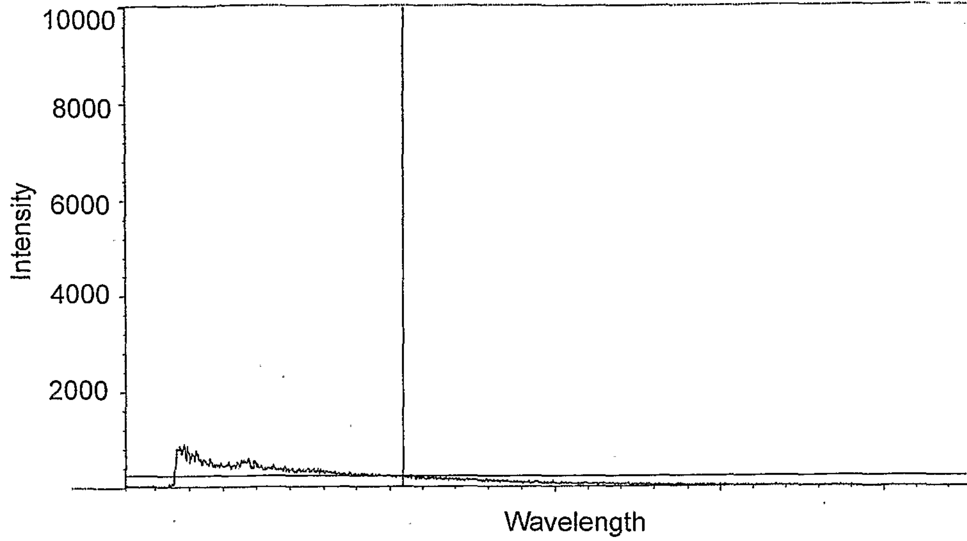



FIGURE 15a

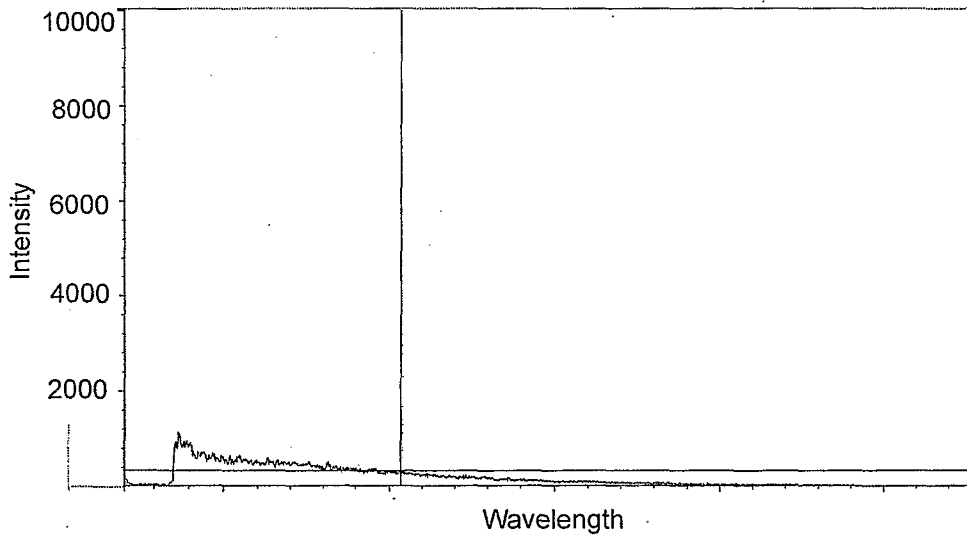



FIGURE 15b

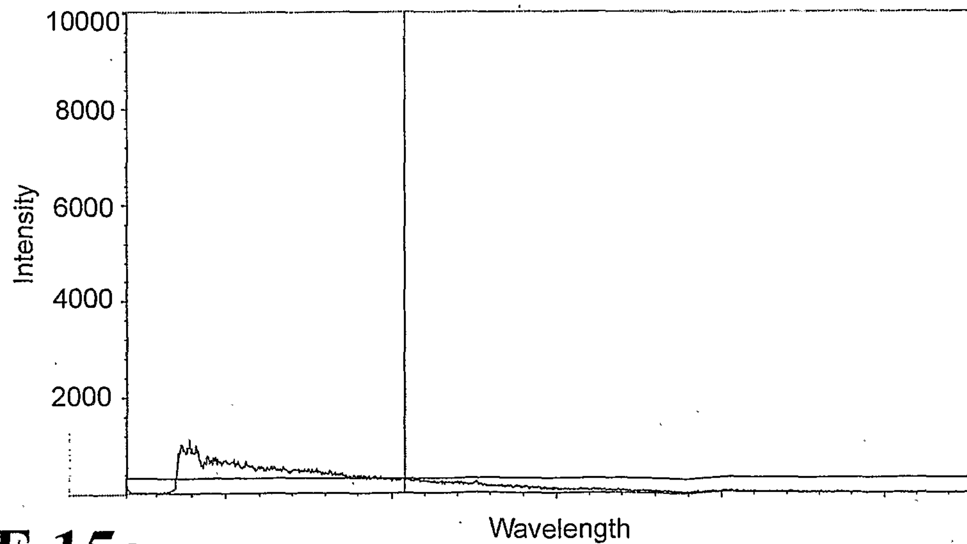



FIGURE 15c

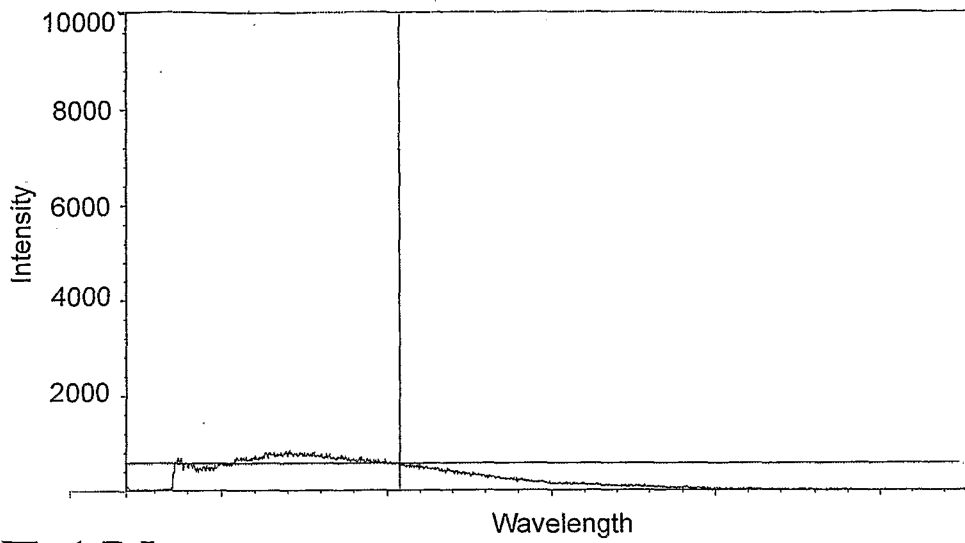



FIGURE 15d

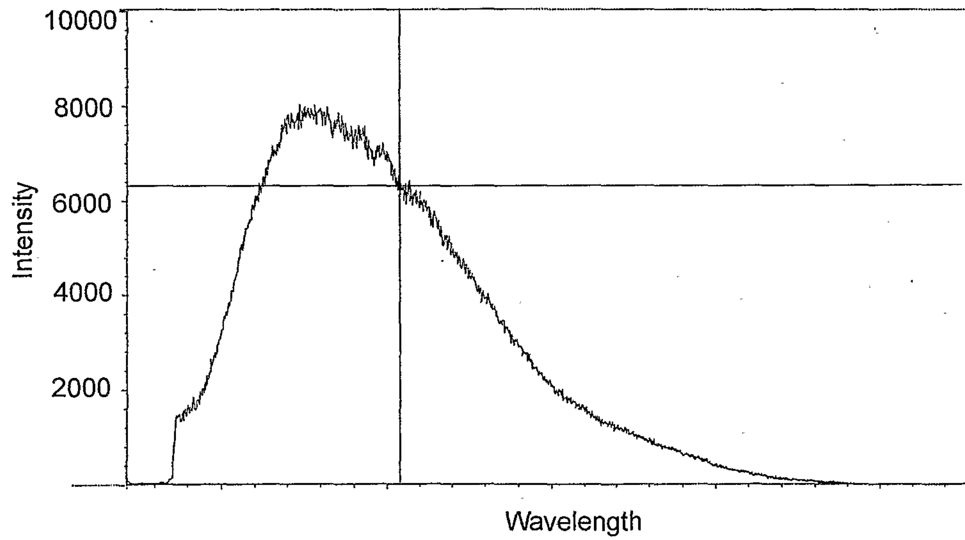



FIGURE 15e

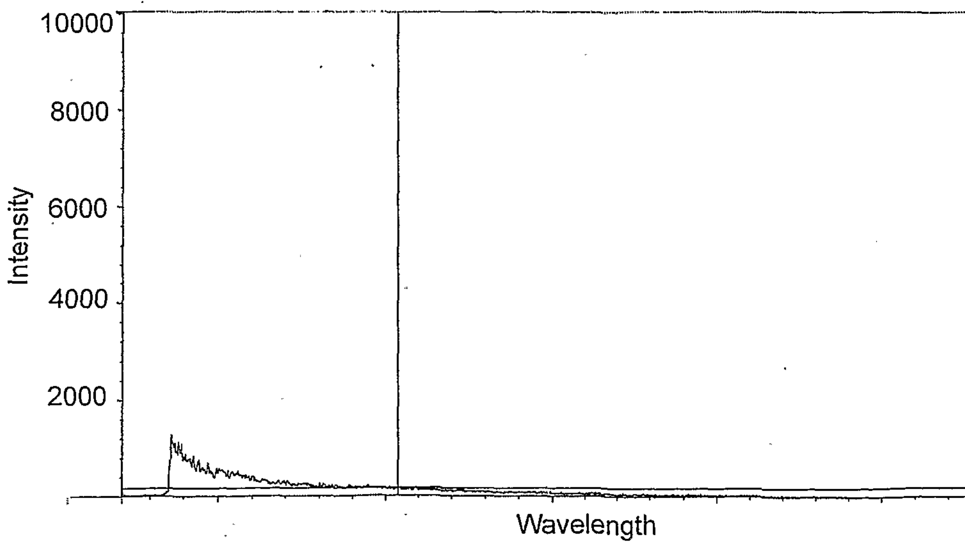



FIGURE 15f

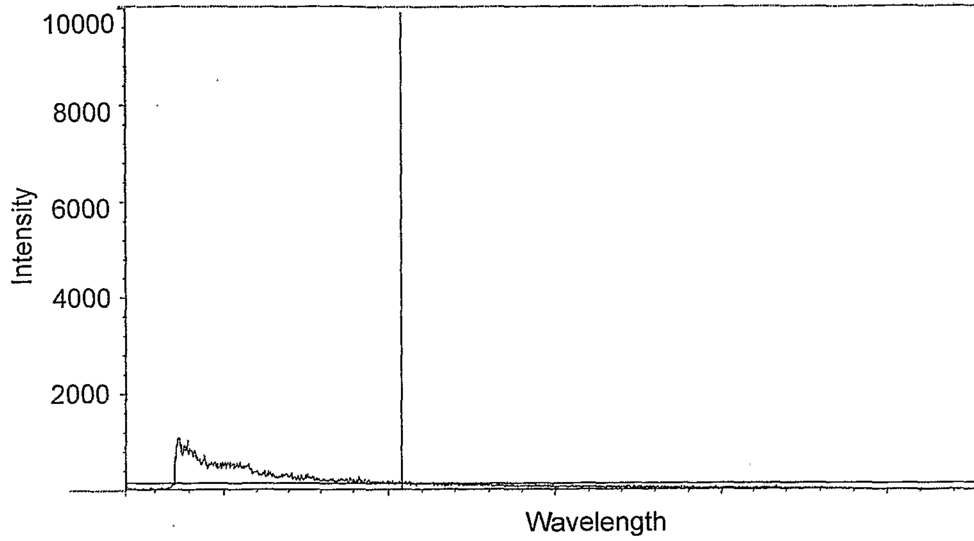



FIGURE 15g

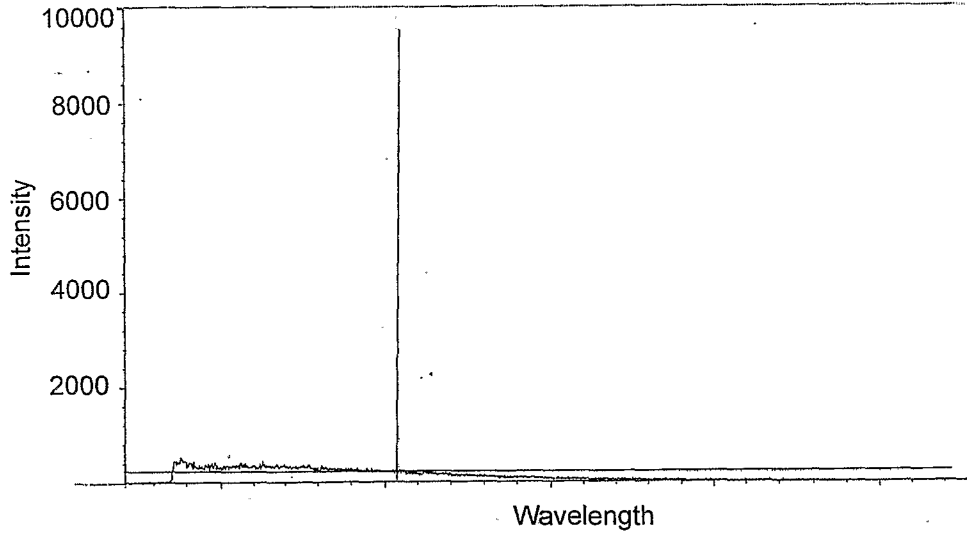



FIGURE 16a

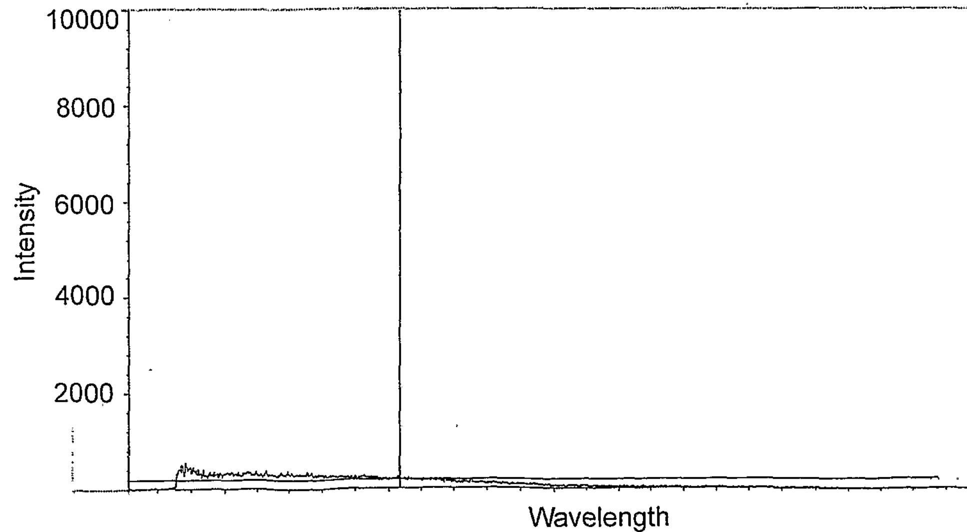



FIGURE 16b

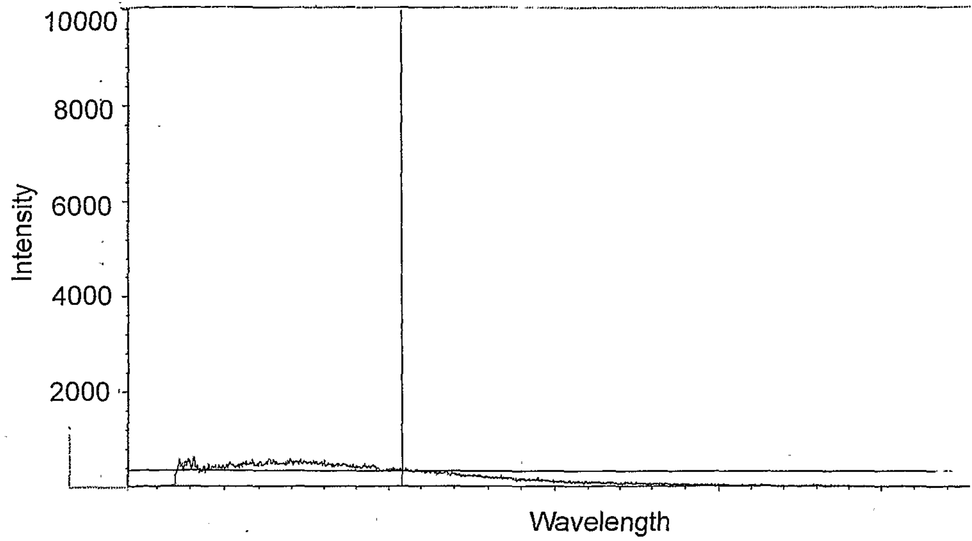



FIGURE 16c

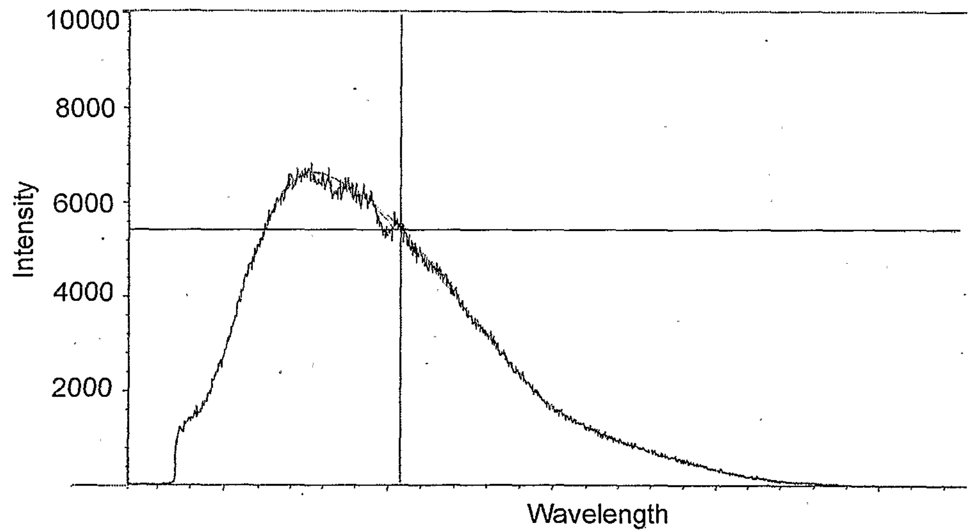



FIGURE 16d

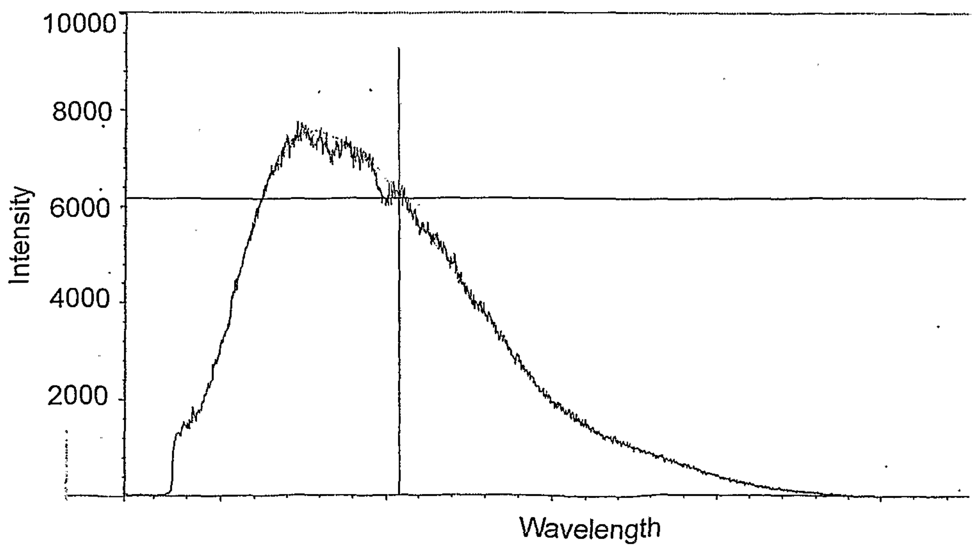



FIGURE 16e

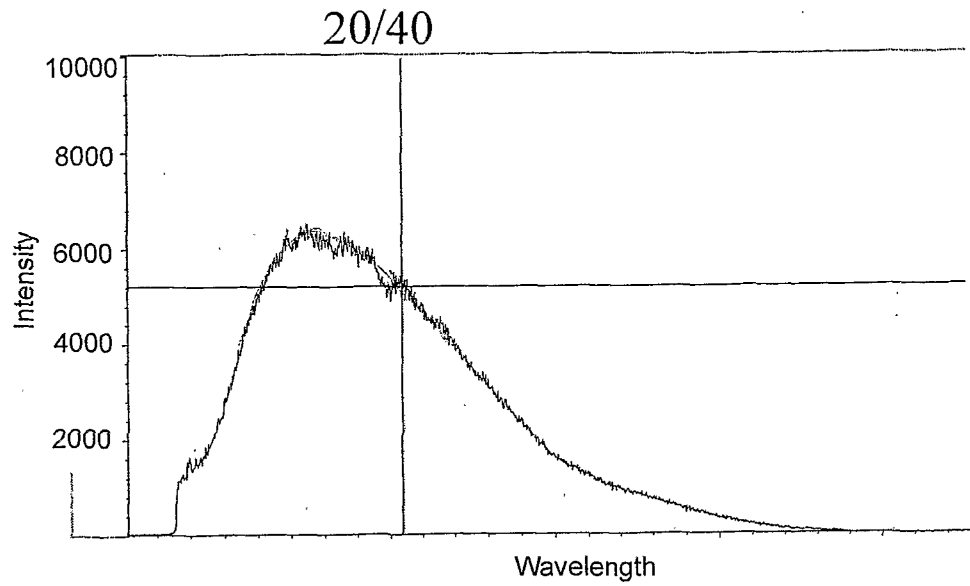



FIGURE 16f

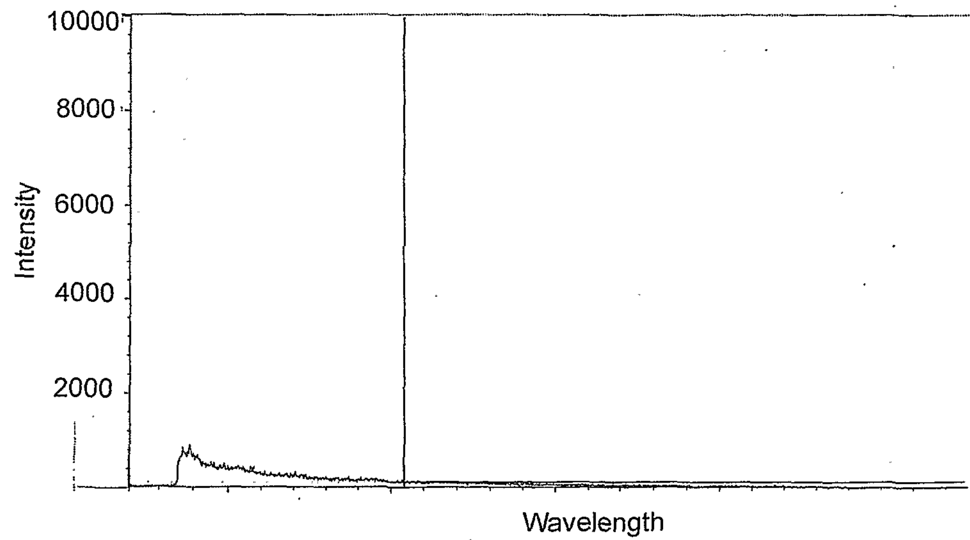



FIGURE 16g

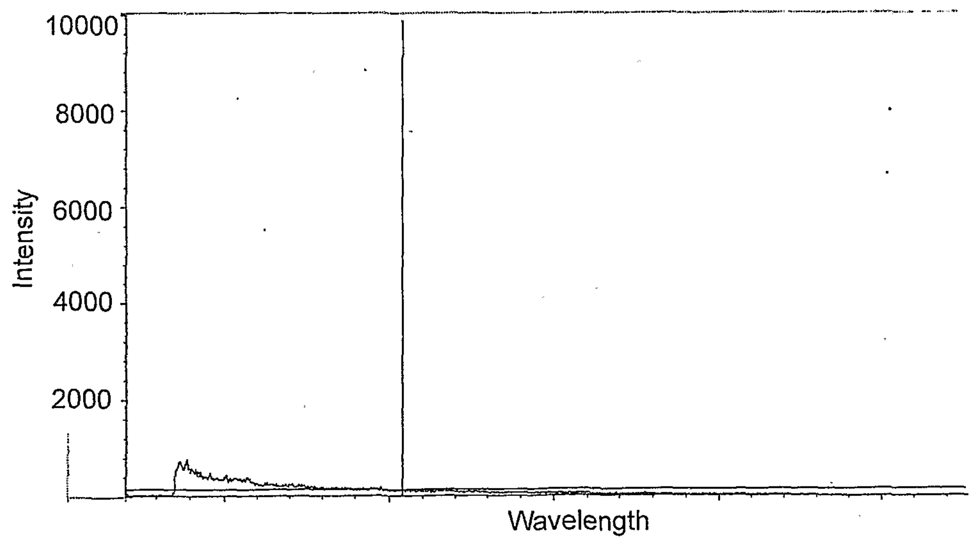
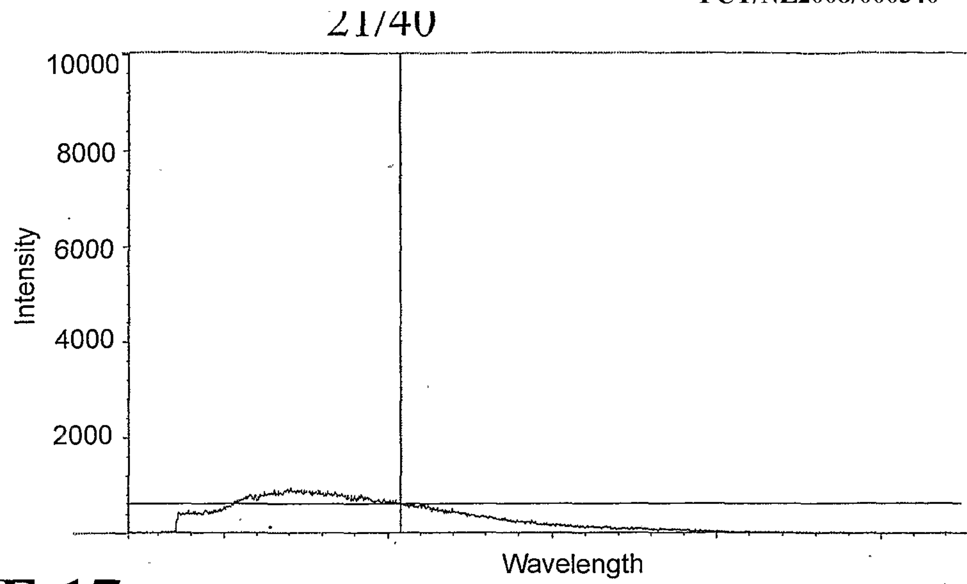
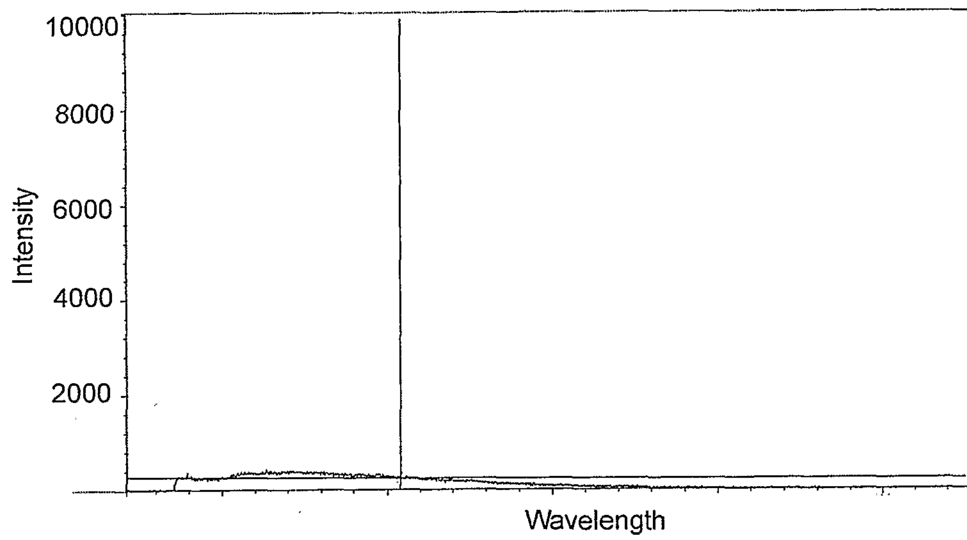


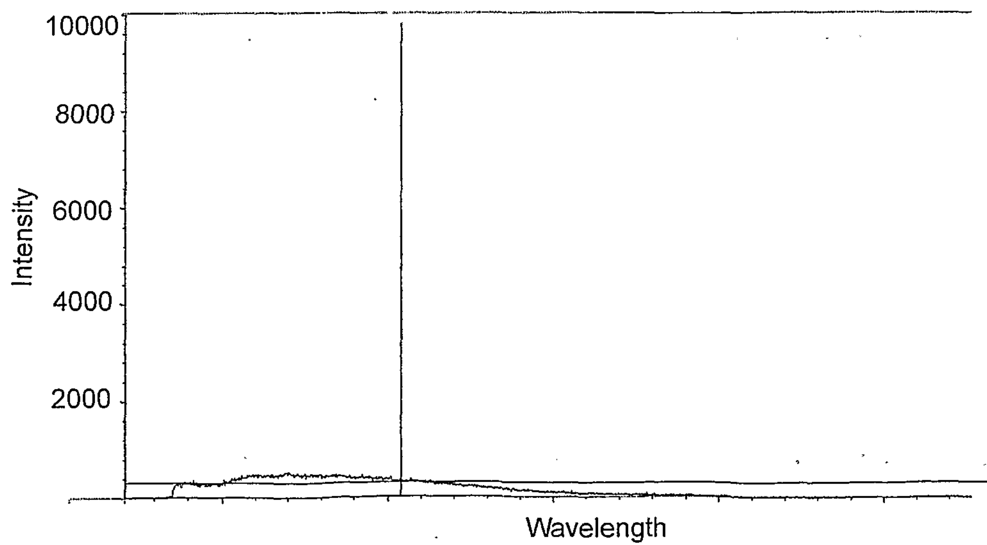

FIGURE 16h




# FIGURE 17a




# FIGURE 17b




# FIGURE 17c

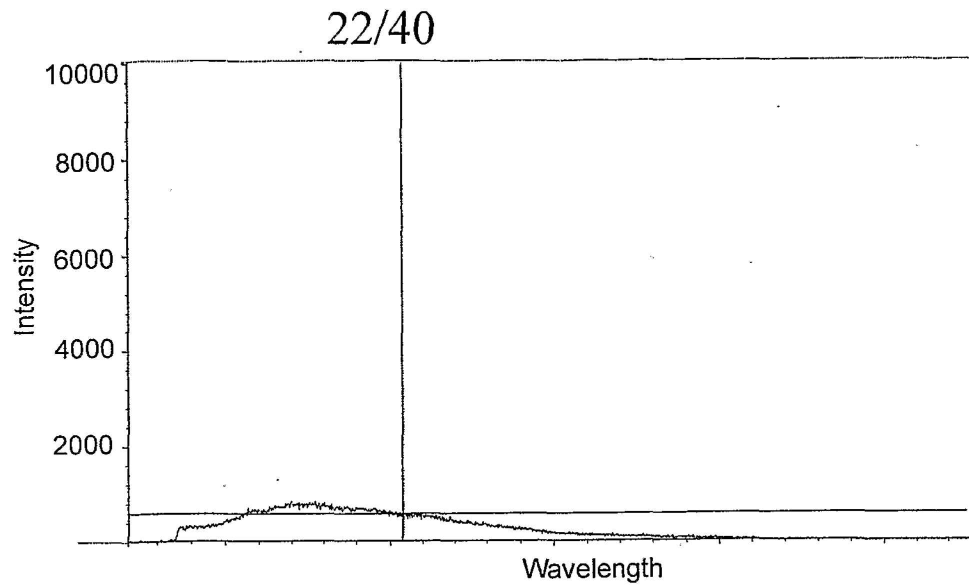



FIGURE 17d

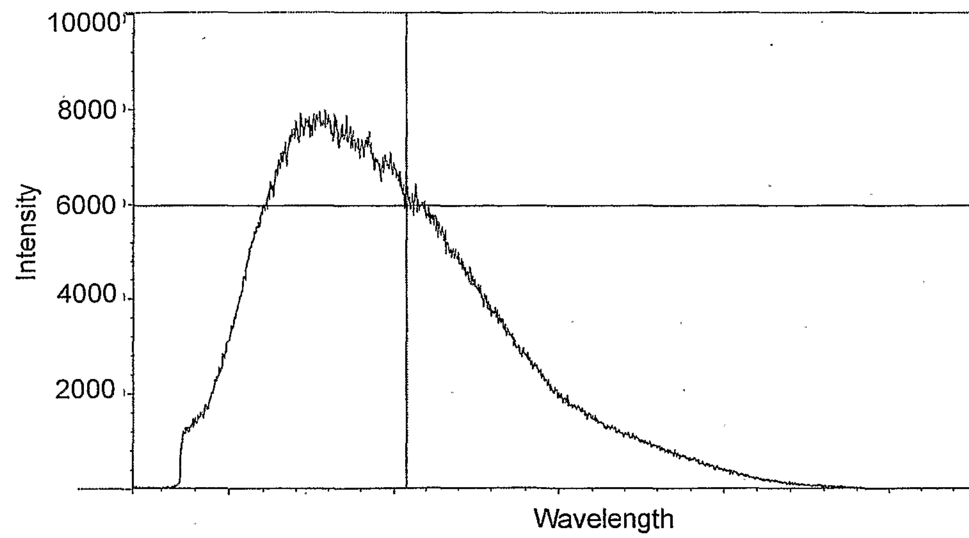



FIGURE 17e

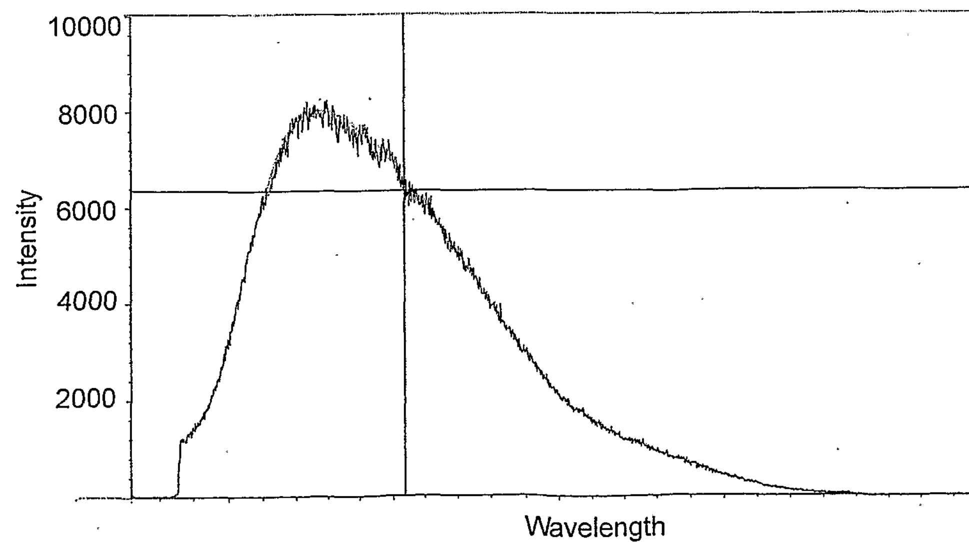



FIGURE 17f

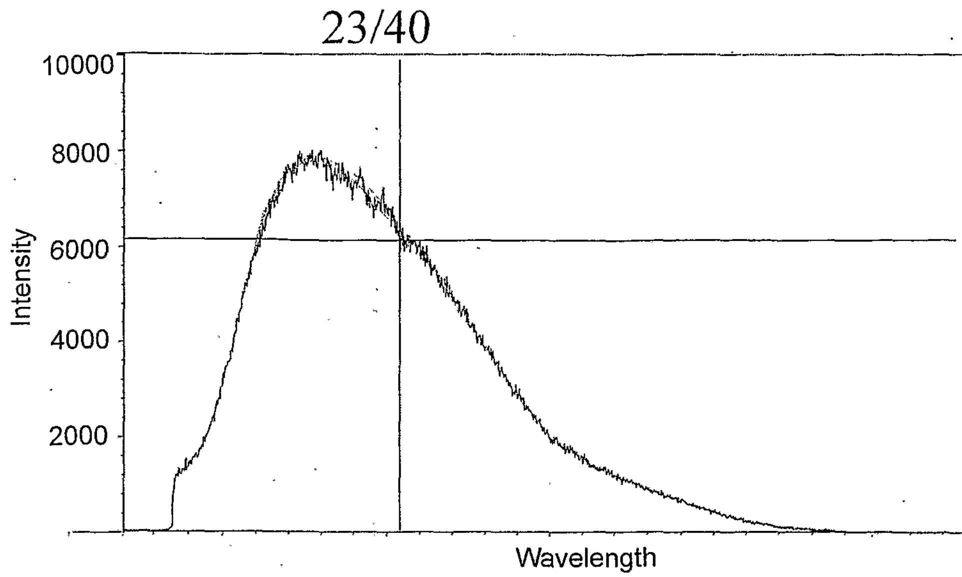



FIGURE 17g

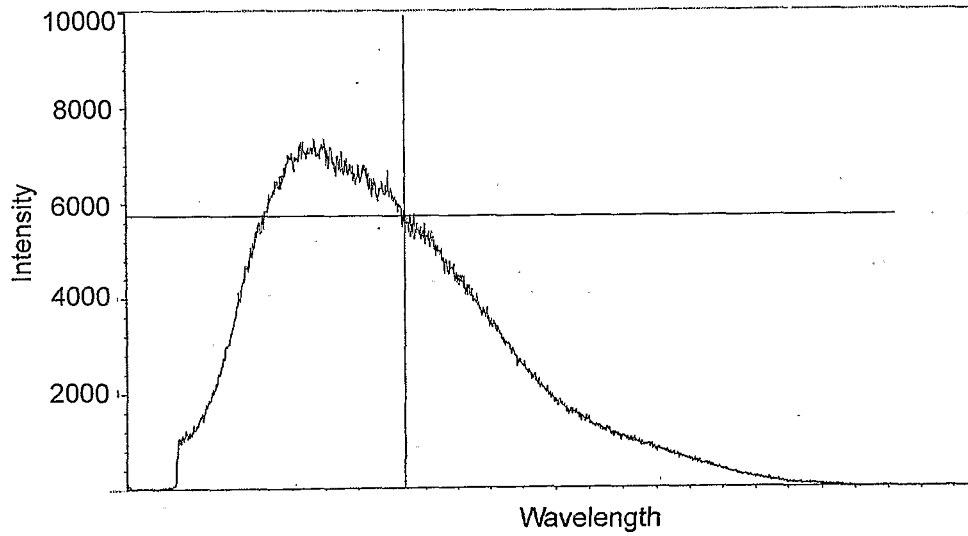



FIGURE 17h

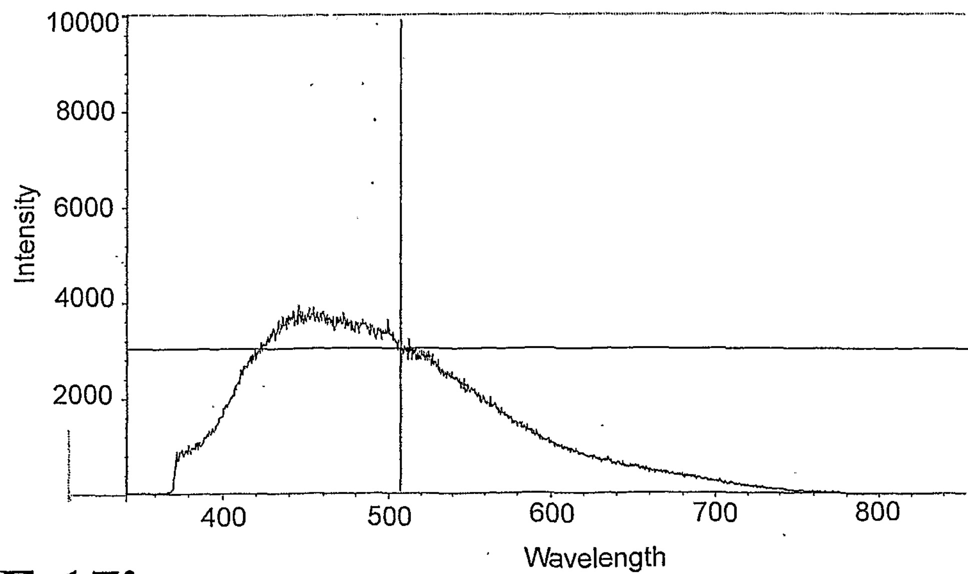
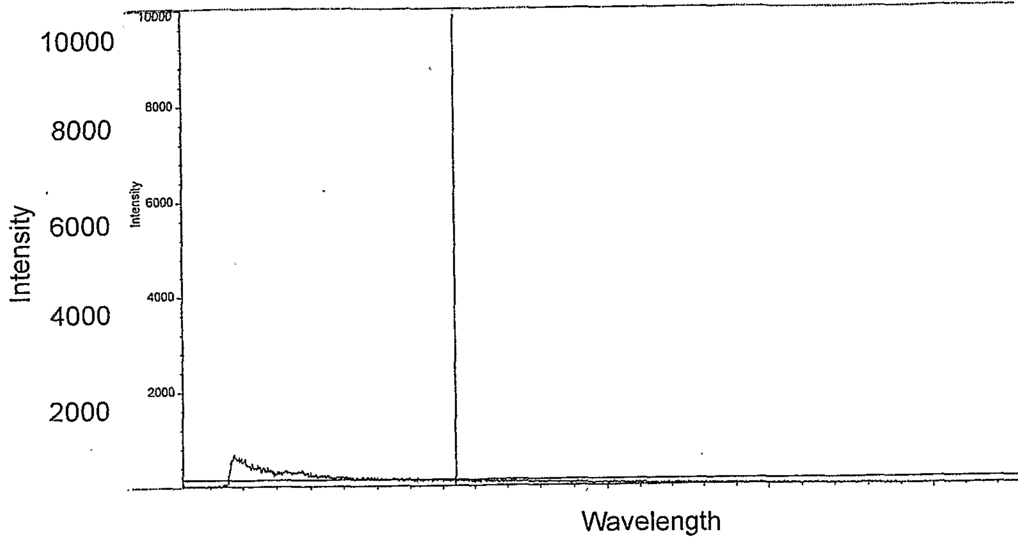
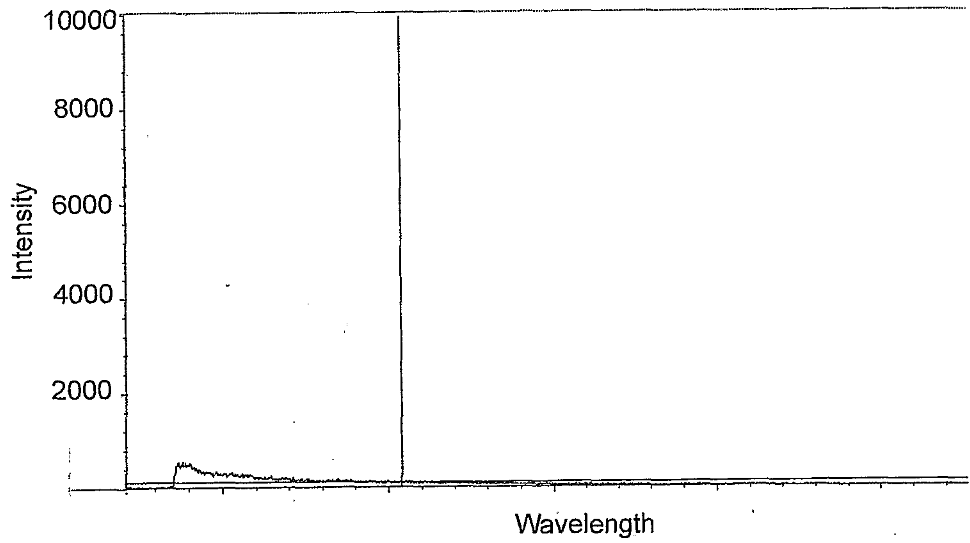


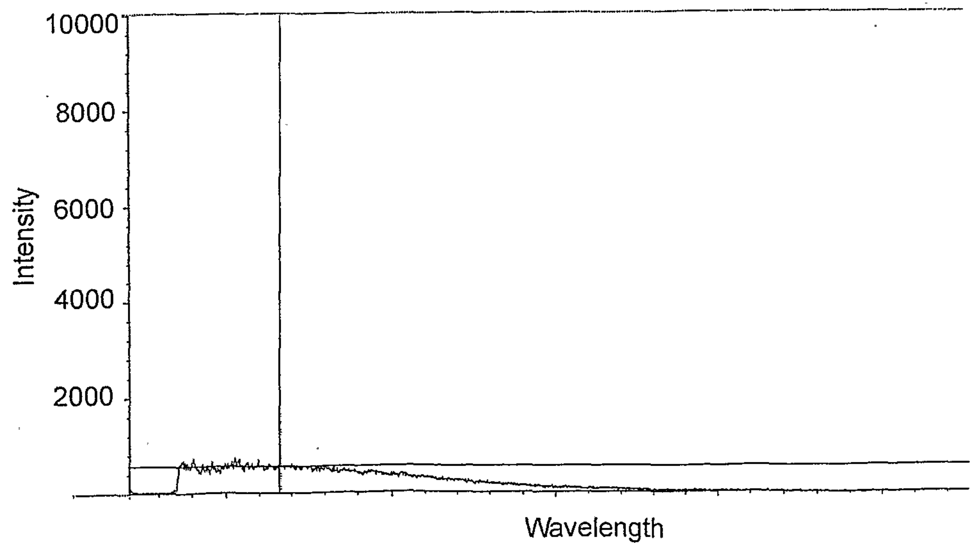

FIGURE 17i




# FIGURE 17j




# FIGURE 17k




# FIGURE 18a

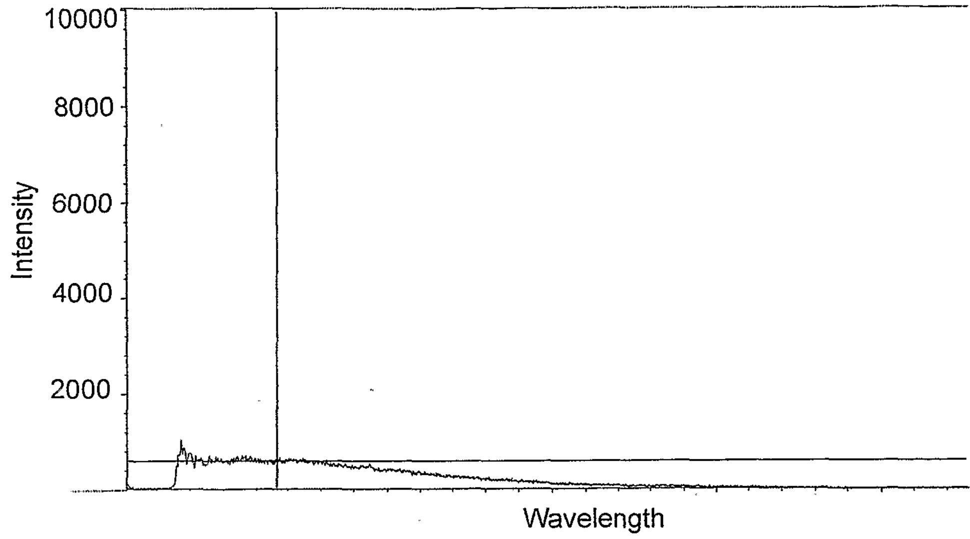



FIGURE 18b

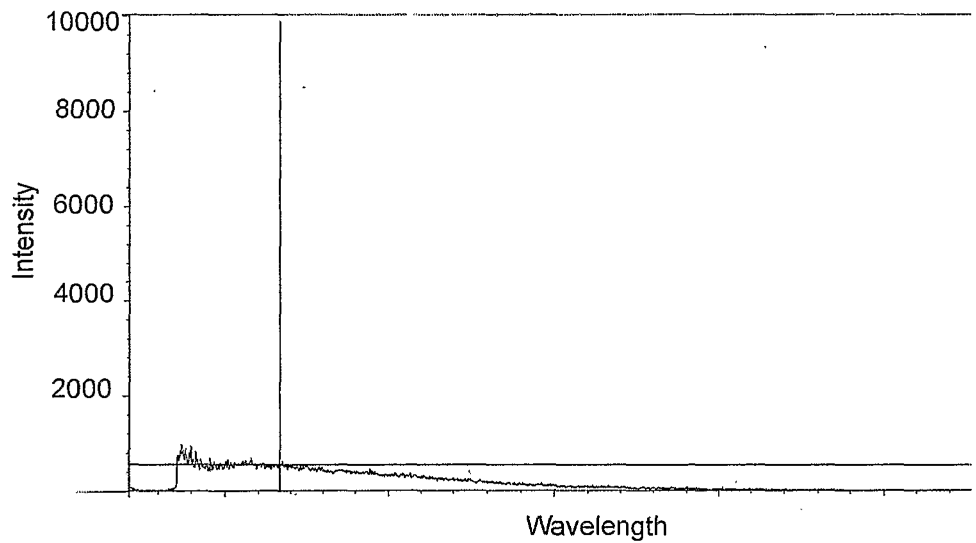



FIGURE 18c

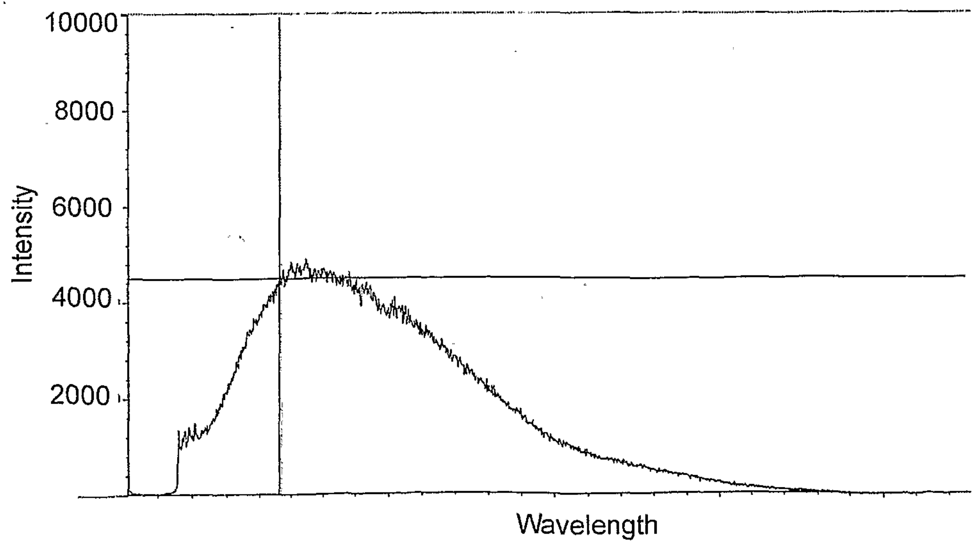
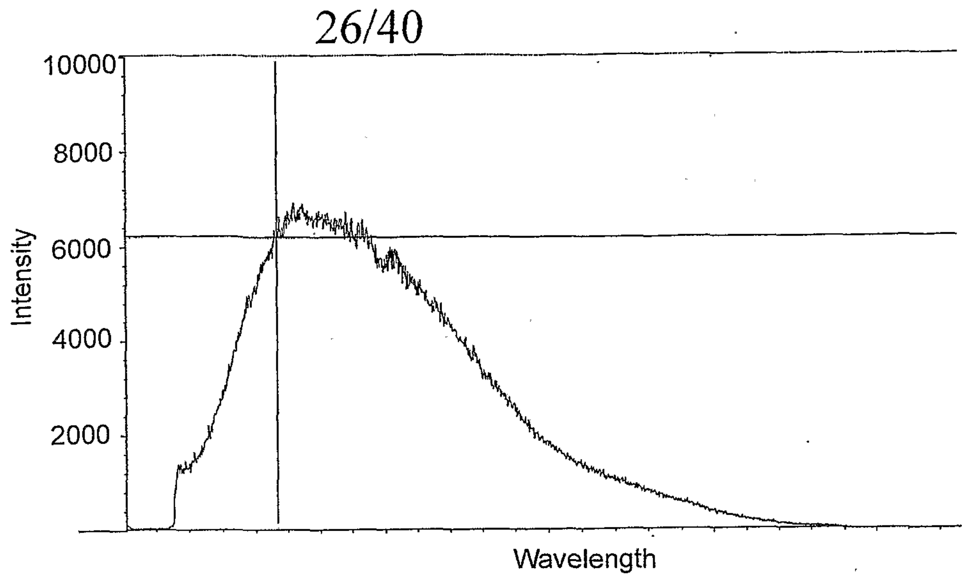
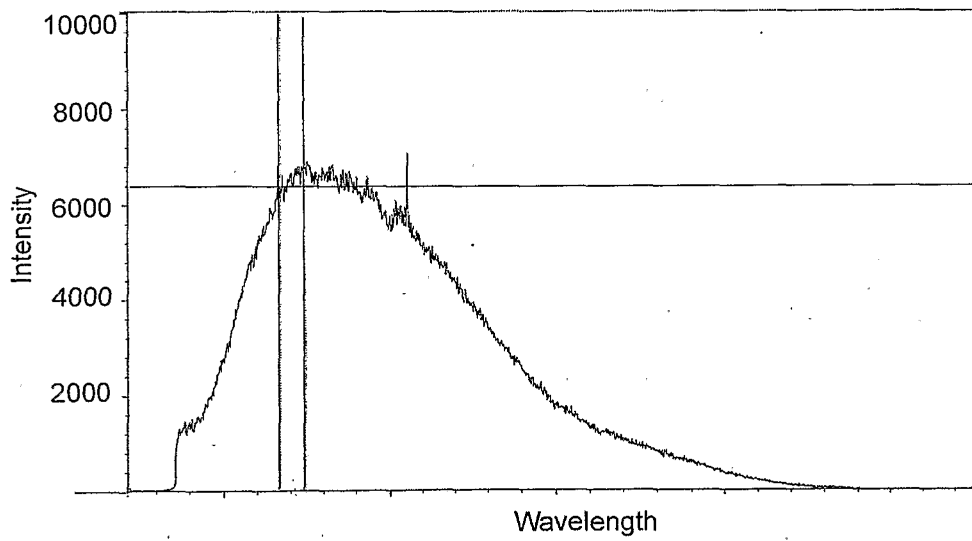


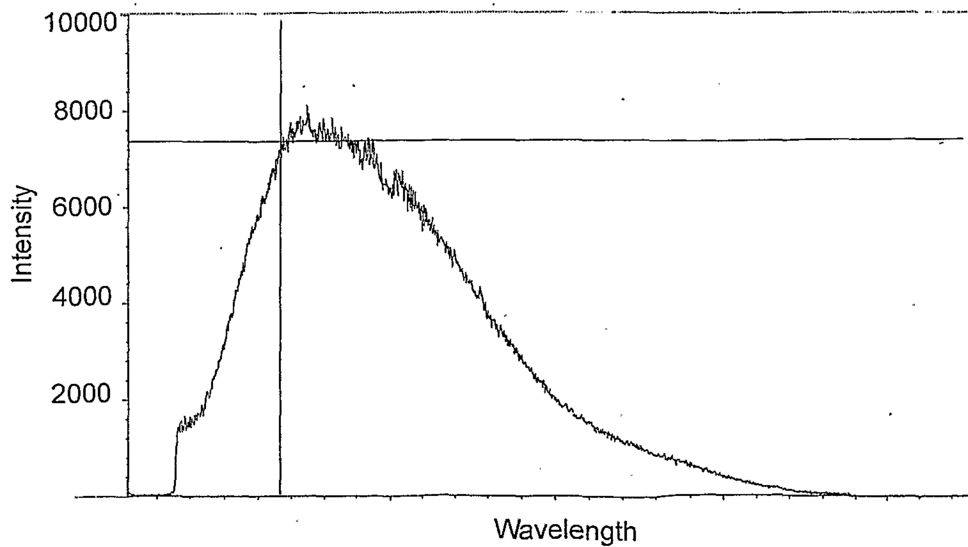

FIGURE 18d



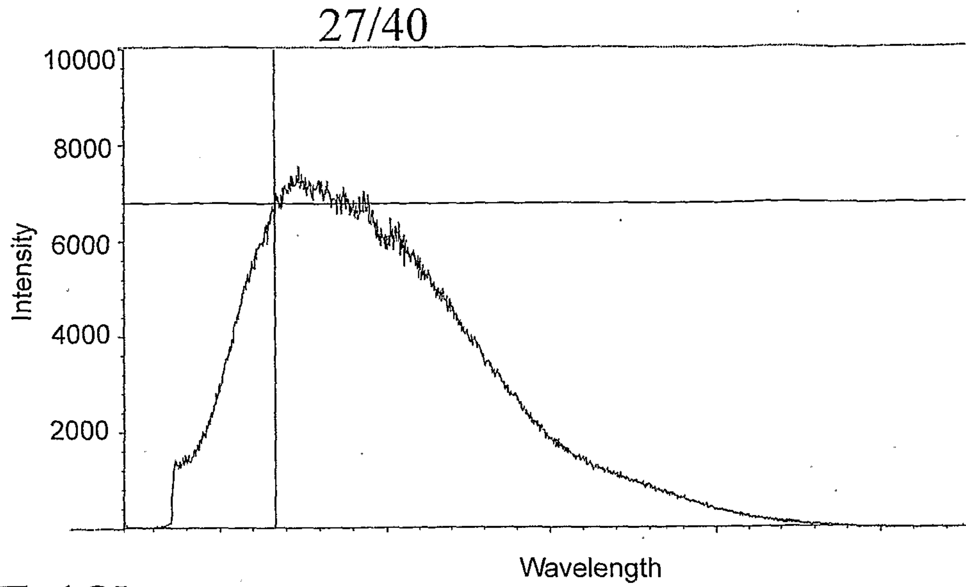

# FIGURE 18e



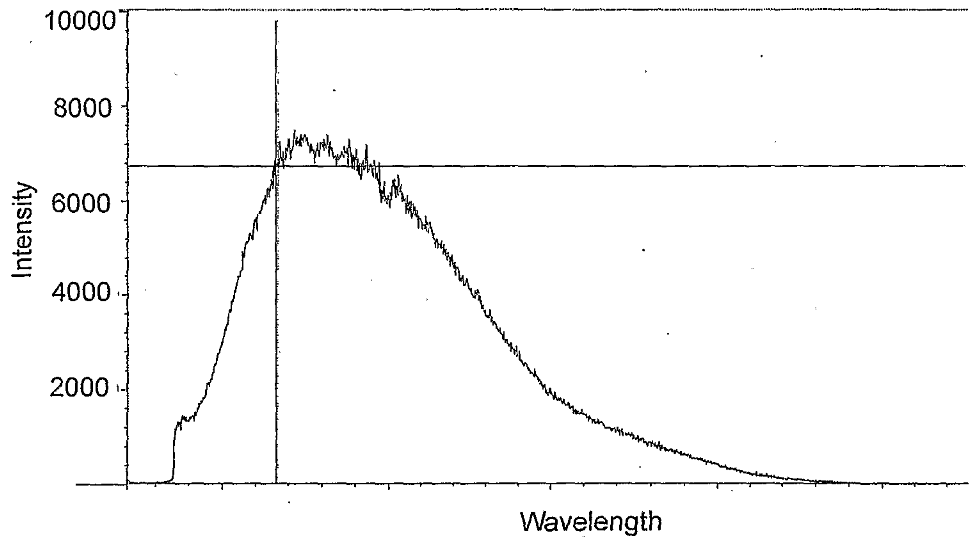

# FIGURE 18f



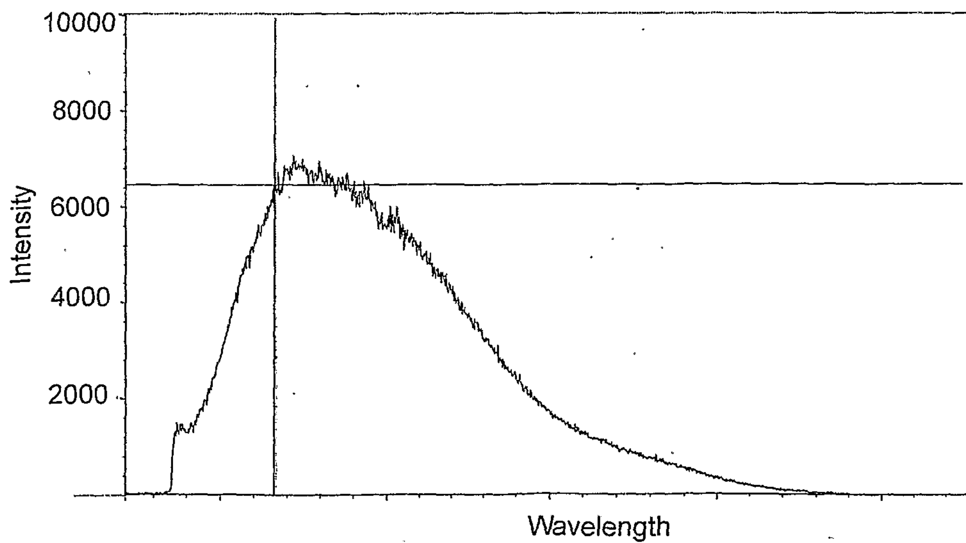

# FIGURE 18g



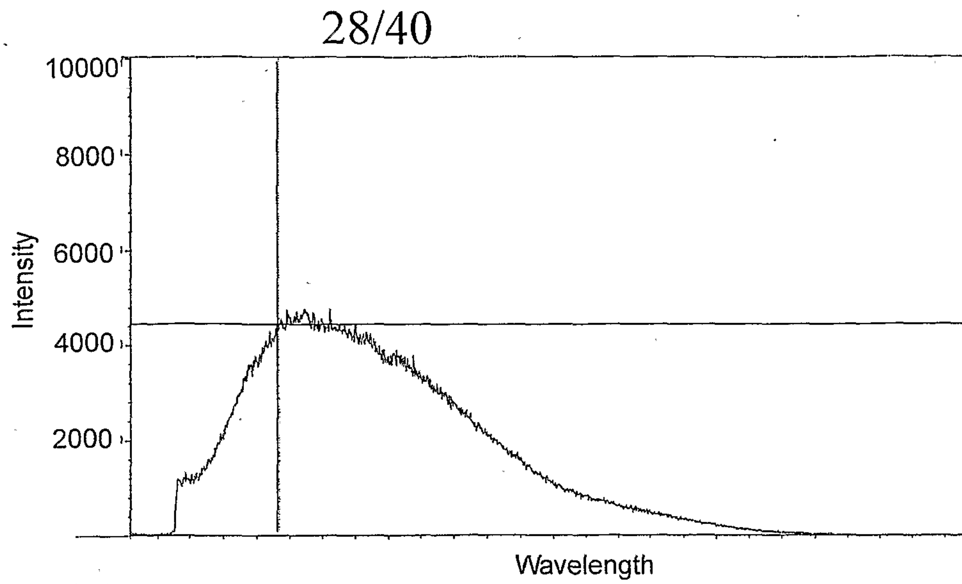

# FIGURE 18h



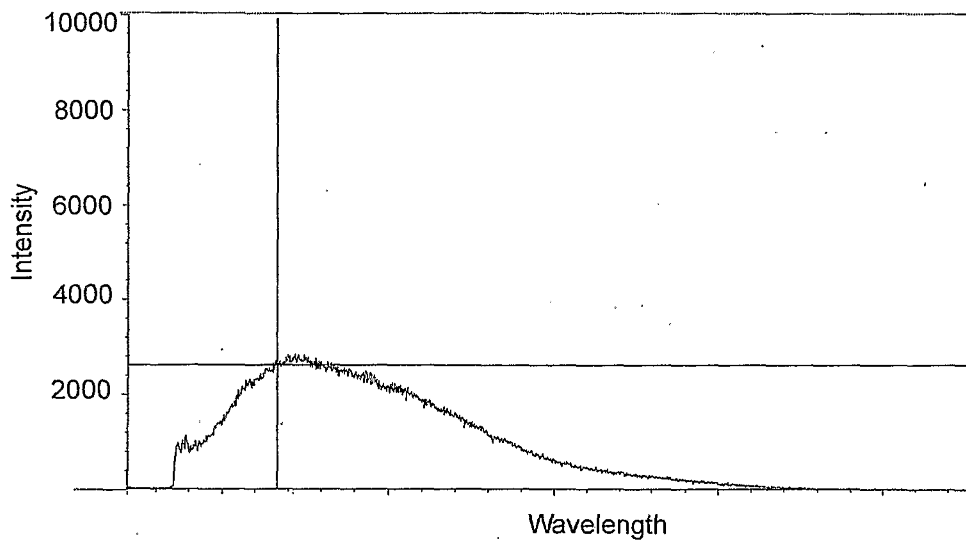

# FIGURE 18i



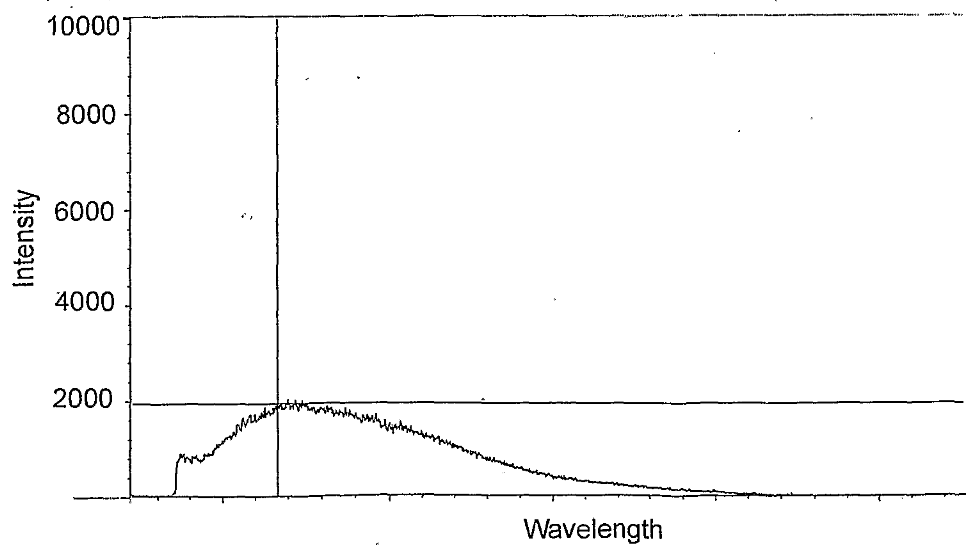

# FIGURE 18j




# FIGURE 18k




# FIGURE 18l




# FIGURE 18m

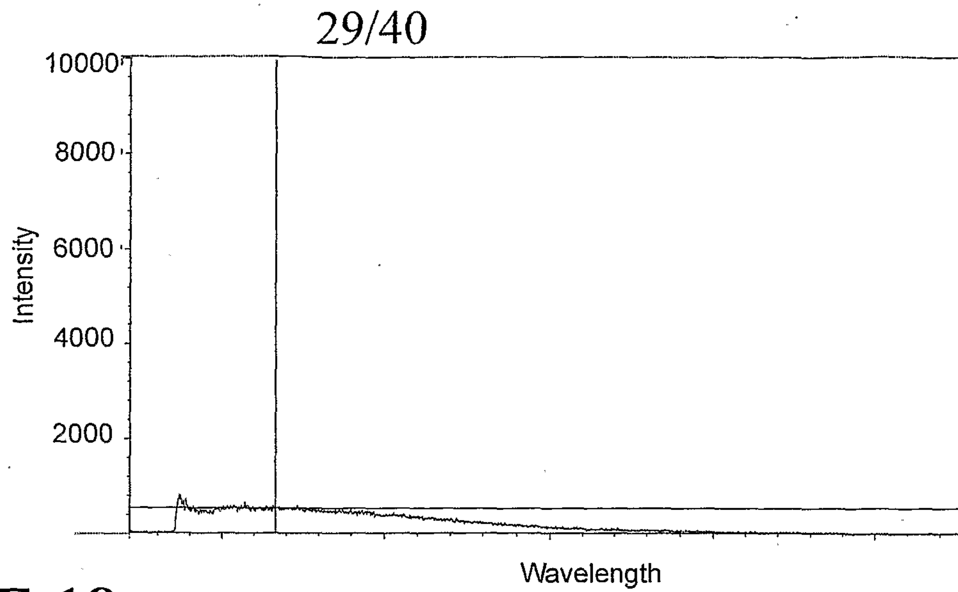



FIGURE 18n

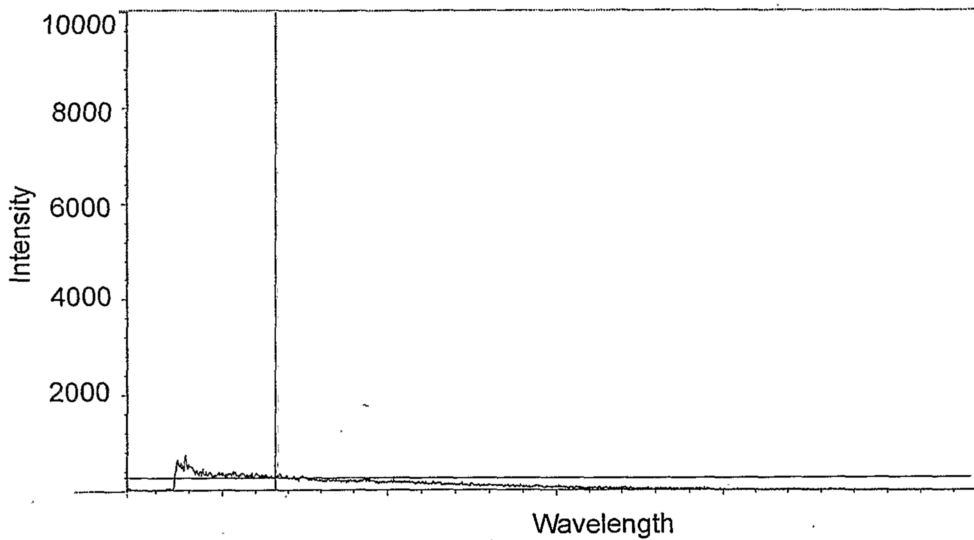



FIGURE 18o

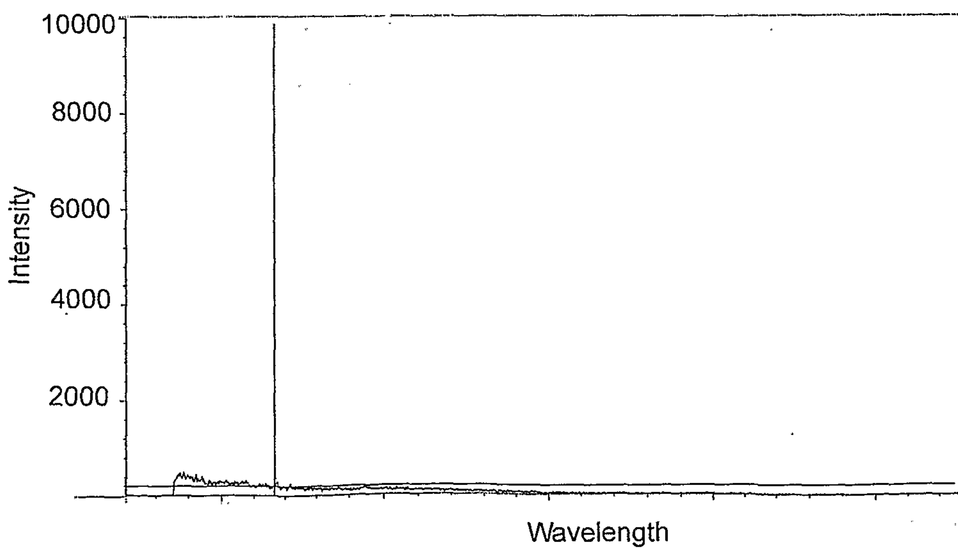
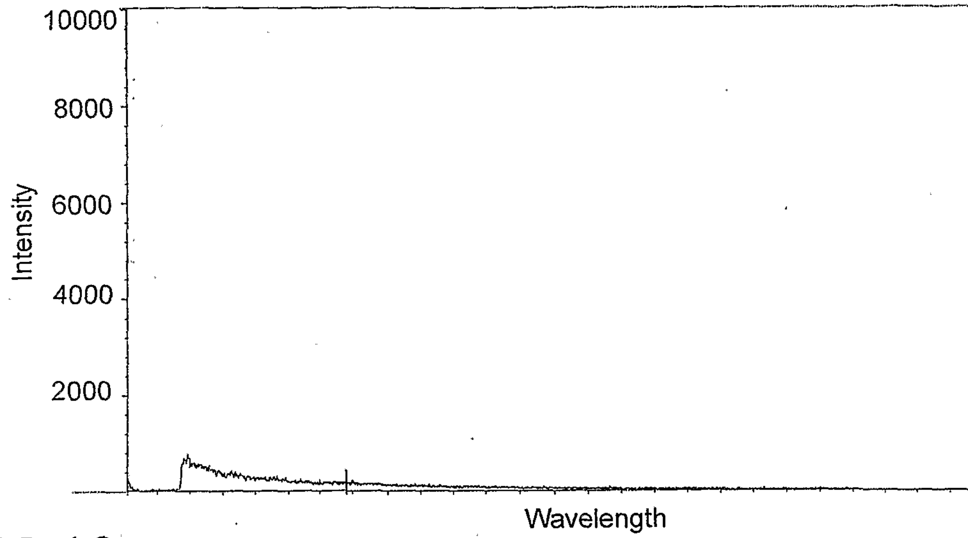
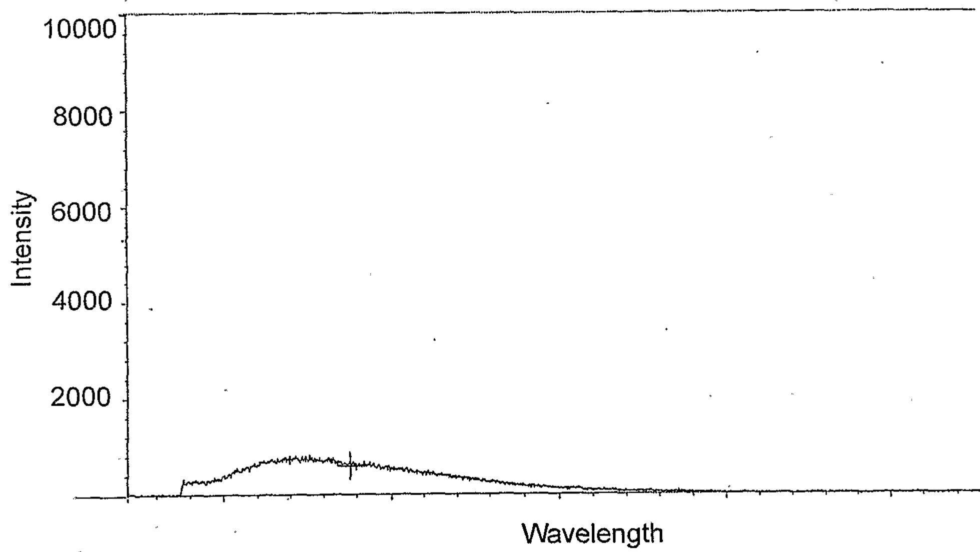


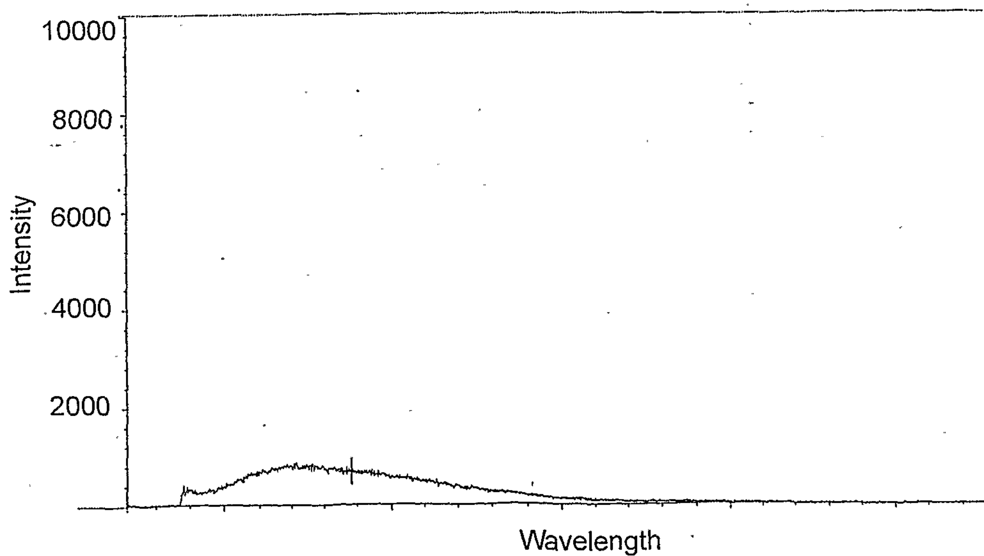

FIGURE 18p



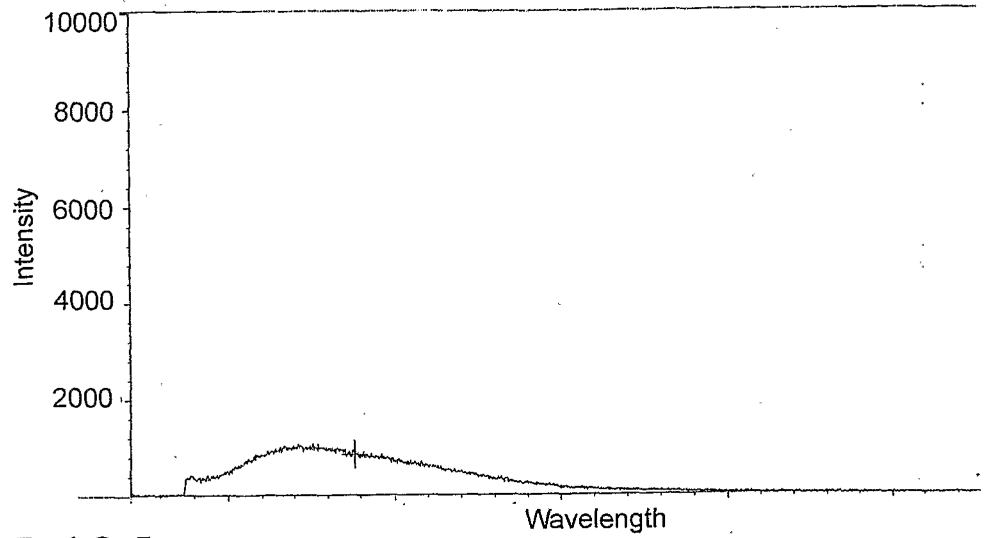

# FIGURE 19a



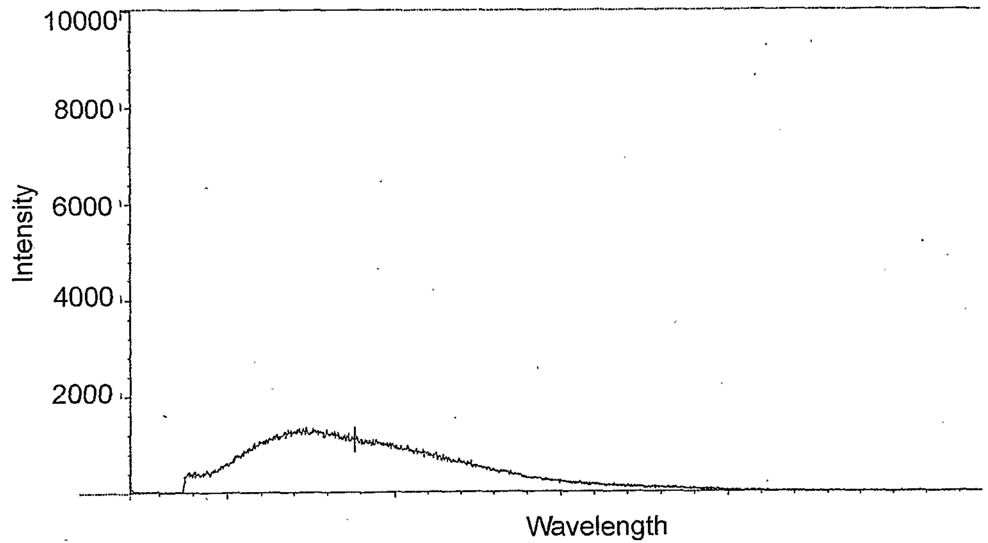

# FIGURE 19b



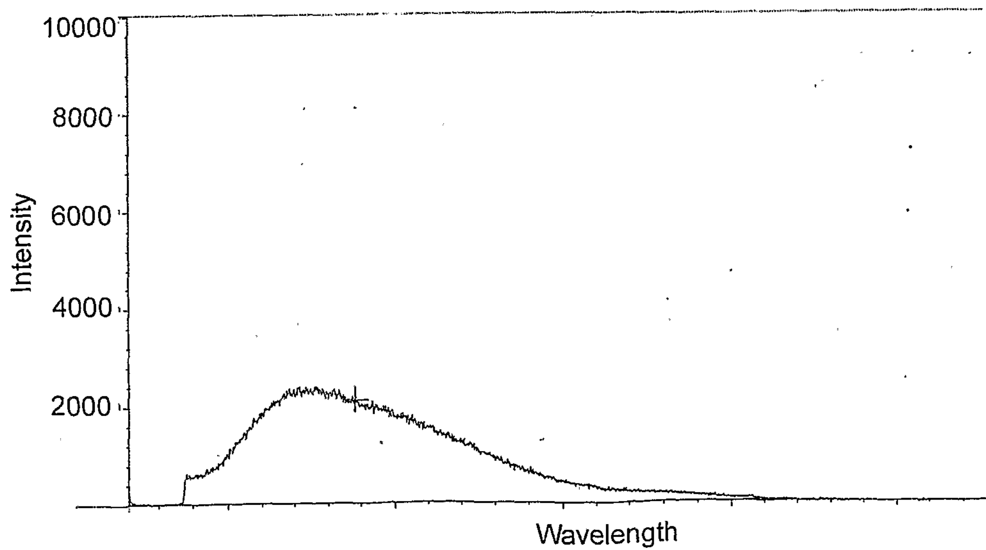

# FIGURE 19c



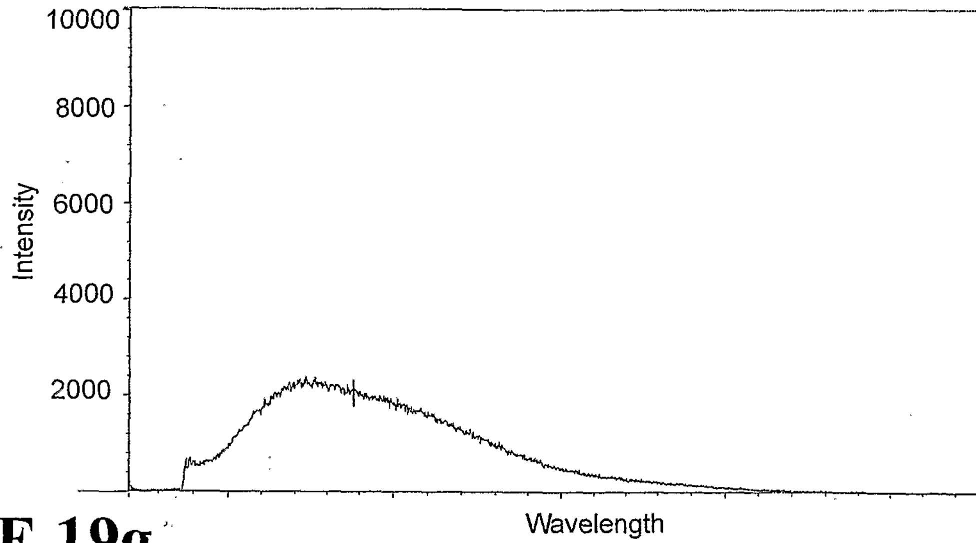

# FIGURE 19d



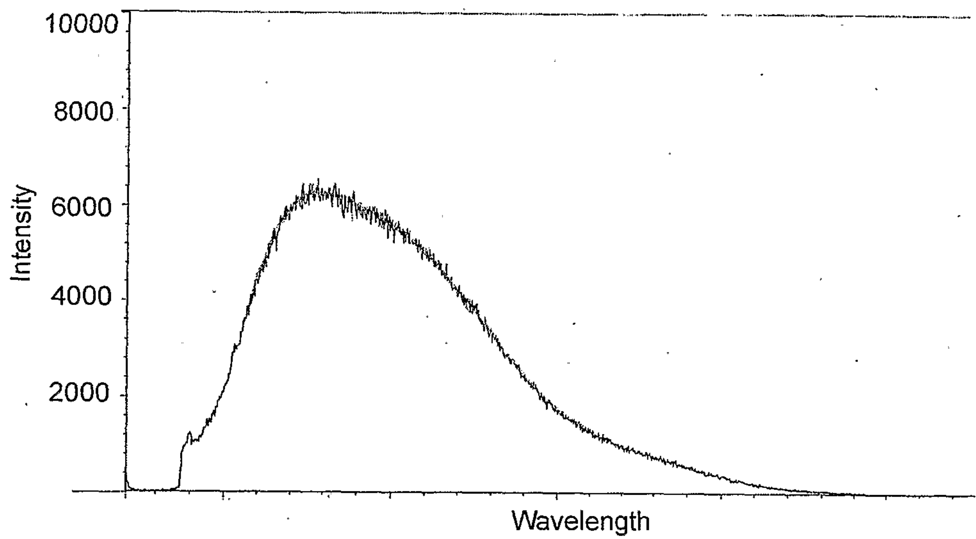

# FIGURE 19e



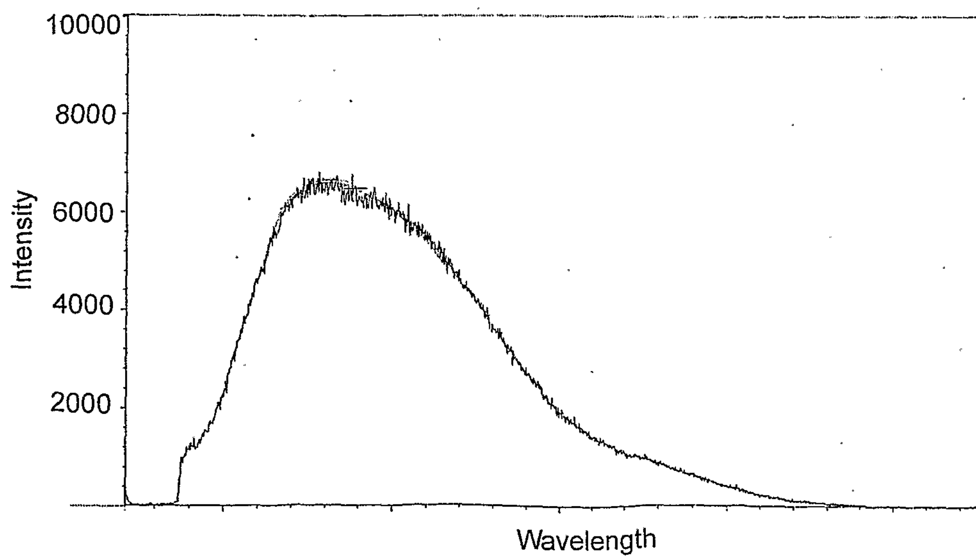

# FIGURE 19f



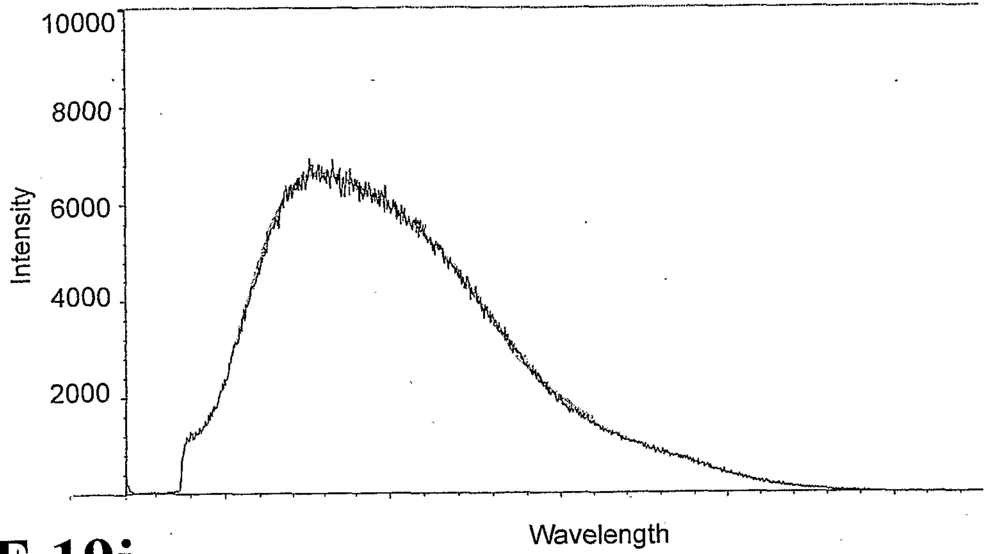

# FIGURE 19g



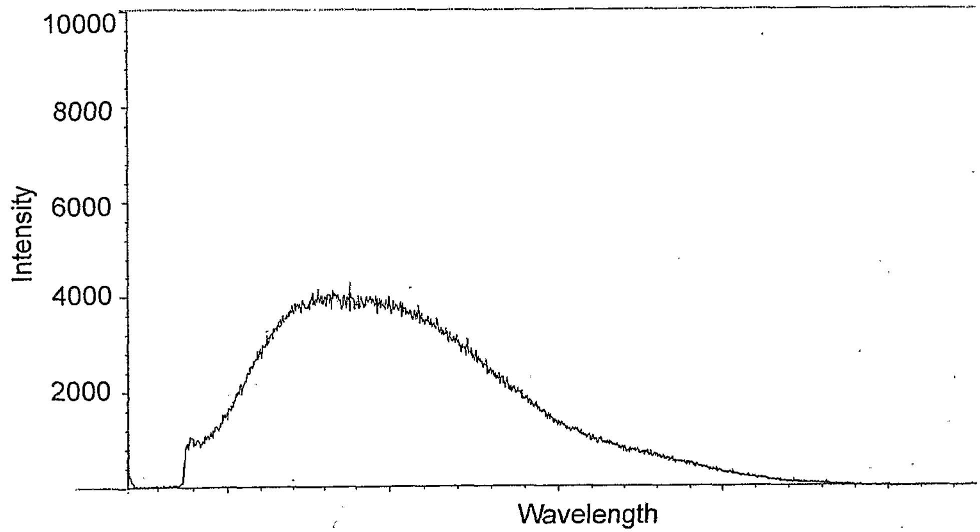

# FIGURE 19h



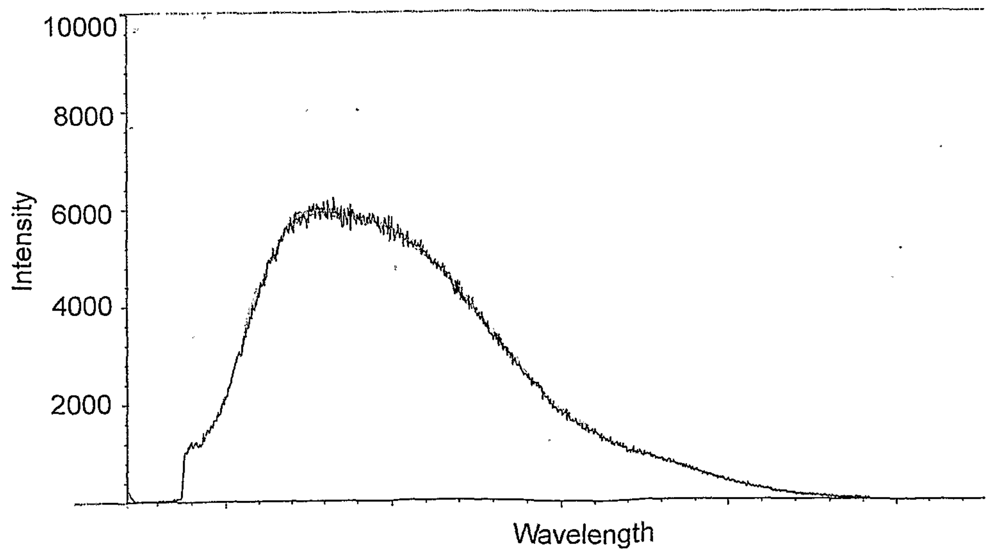

# FIGURE 19i




# FIGURE 19j




# FIGURE 19k




# FIGURE 19l

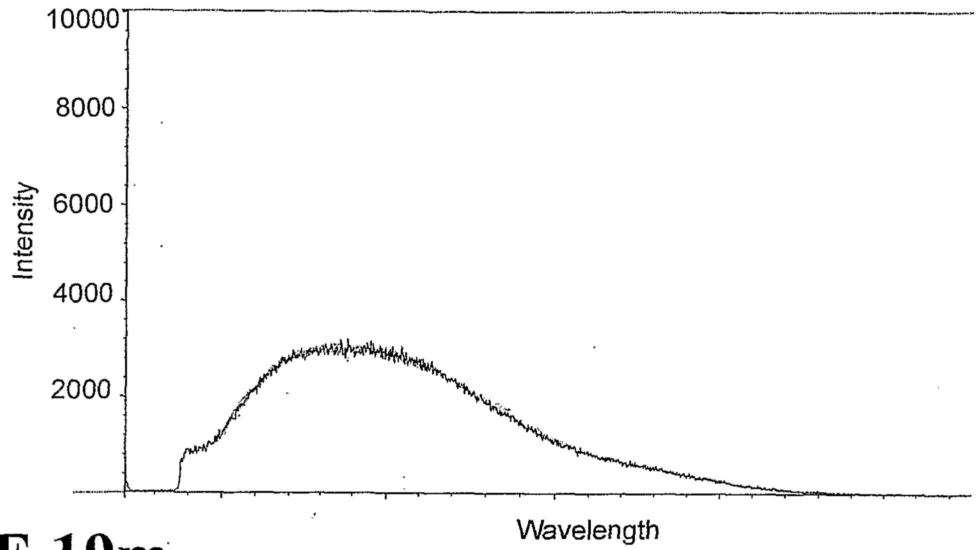



FIGURE 19m

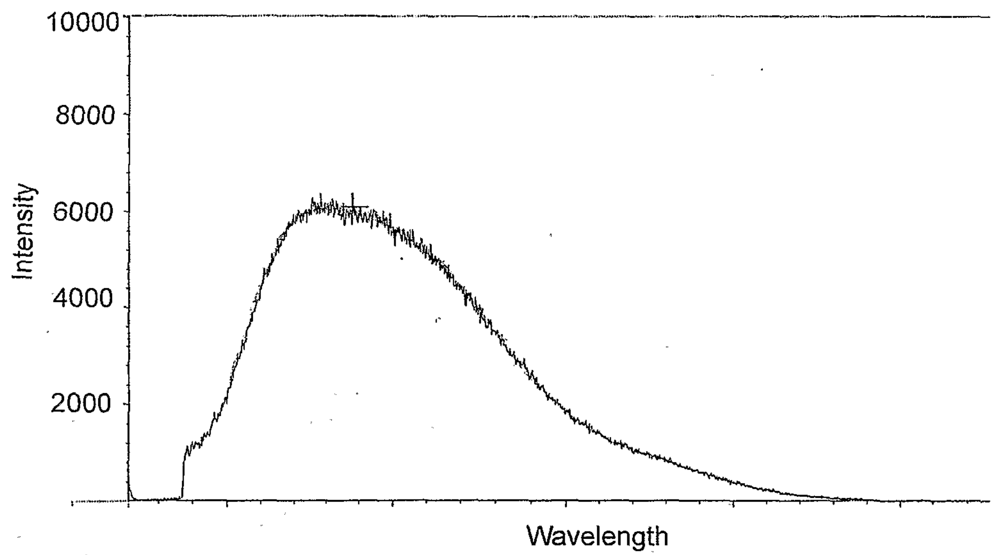



FIGURE 19n

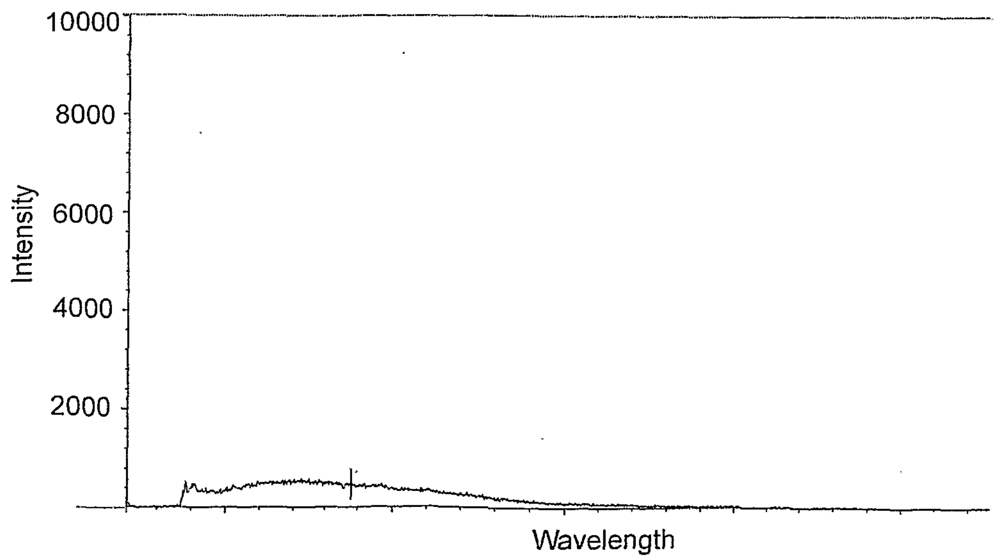



FIGURE 19o

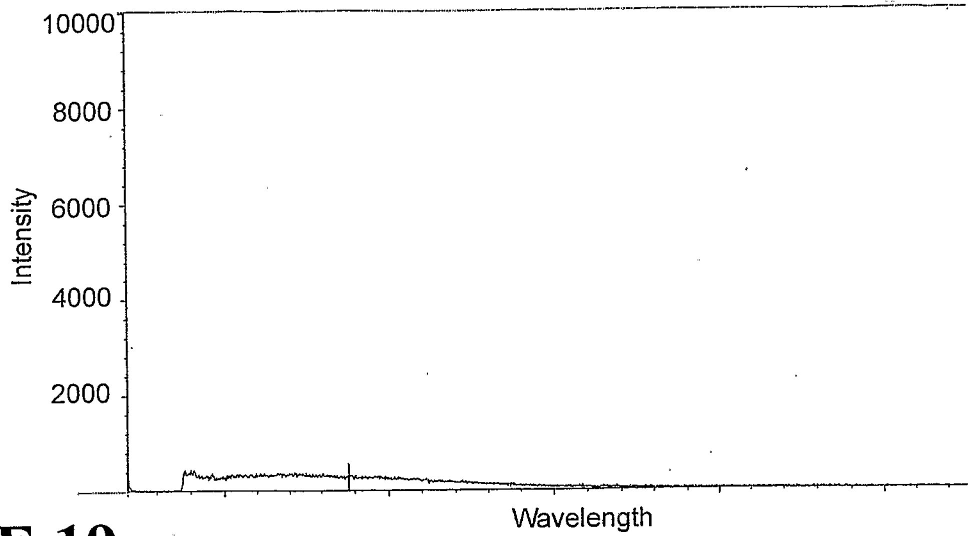



FIGURE 19p

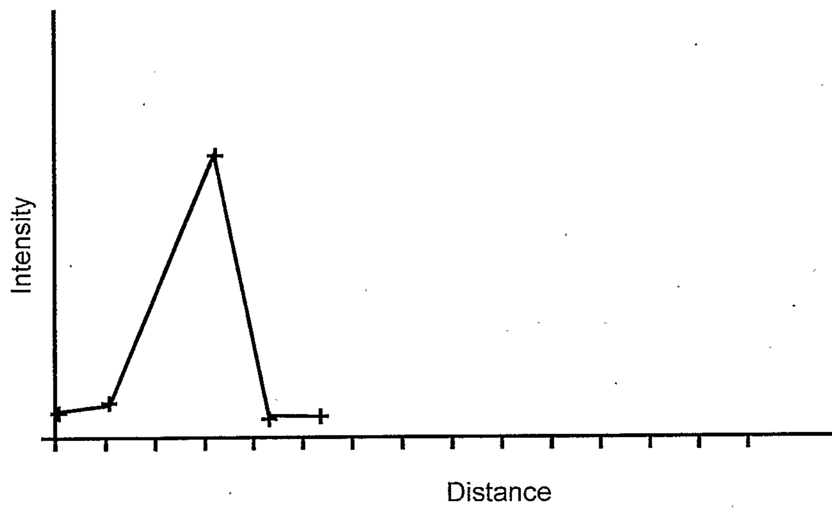



FIGURE 20

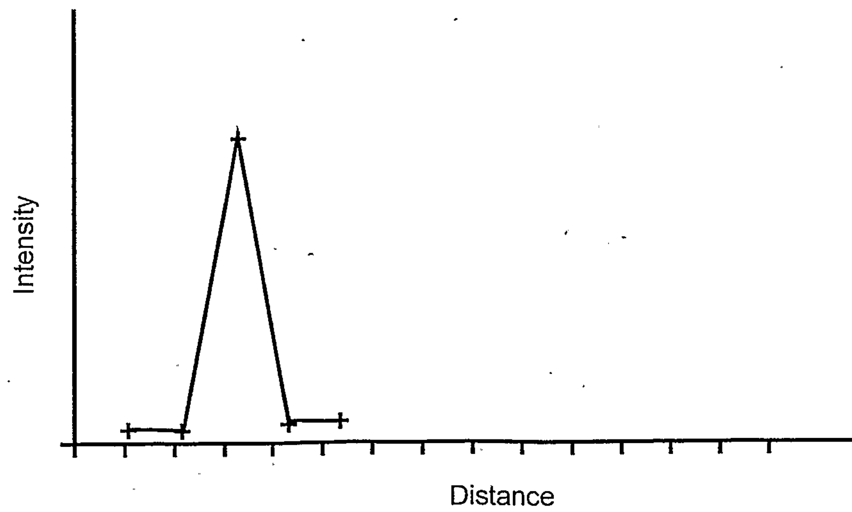



FIGURE 21

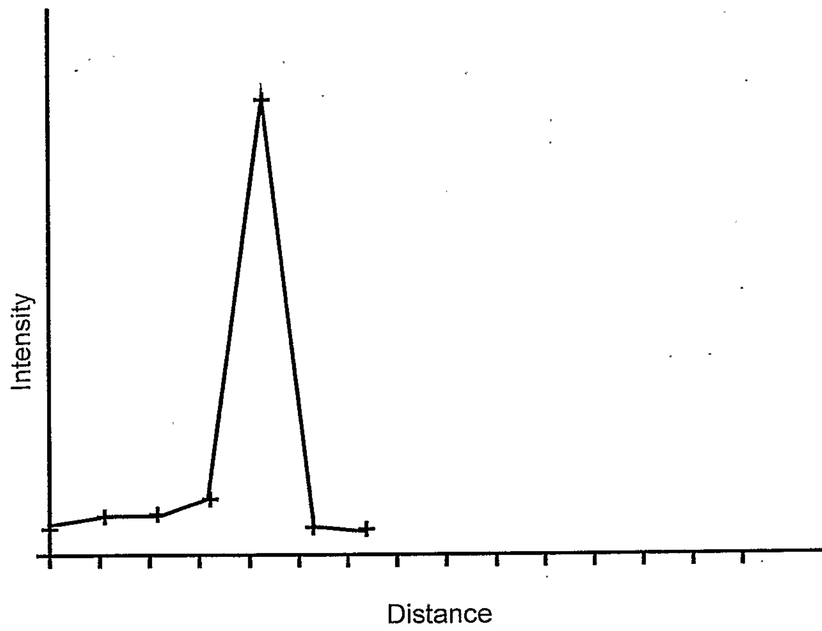



**FIGURE 22**

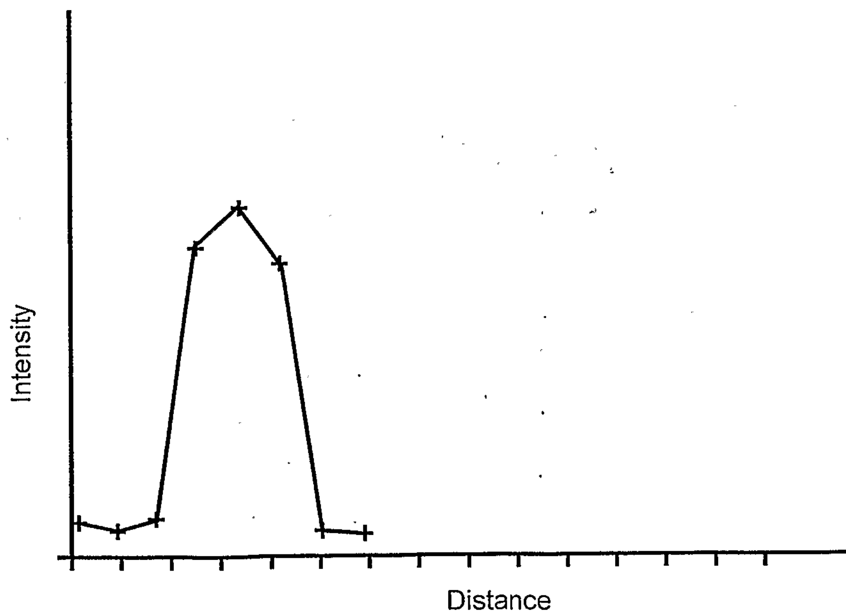



**FIGURE 23**

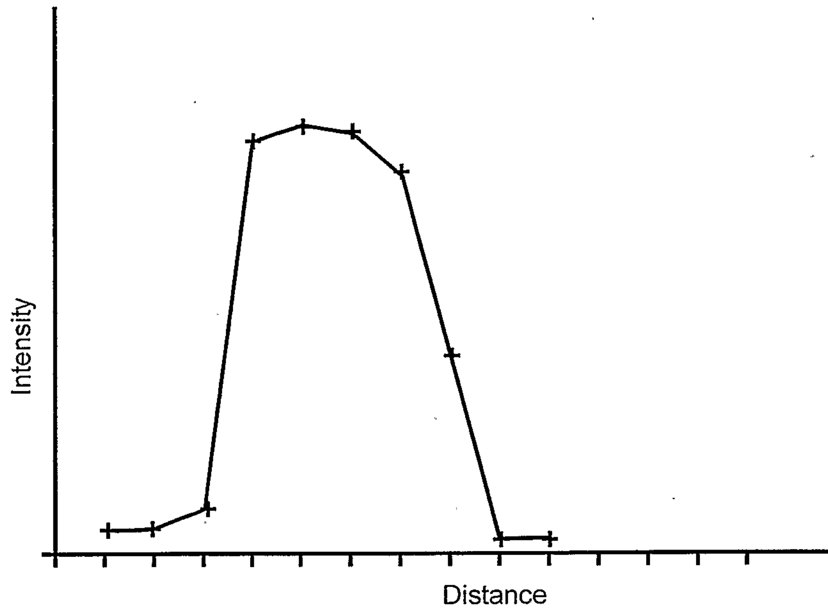



FIGURE 24

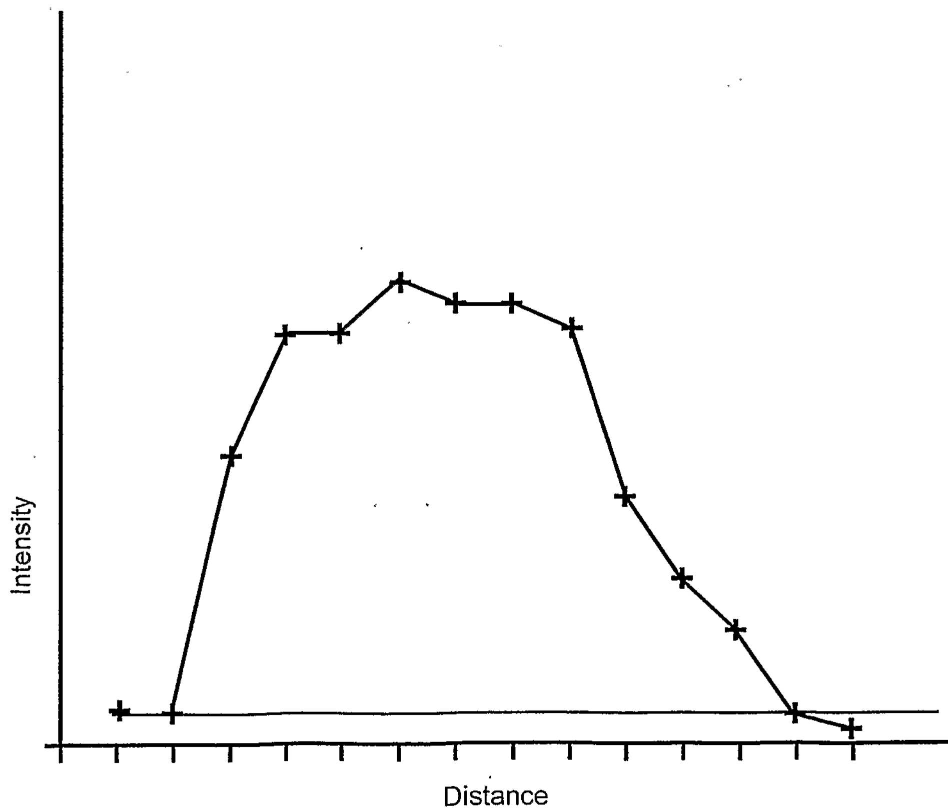



FIGURE 25

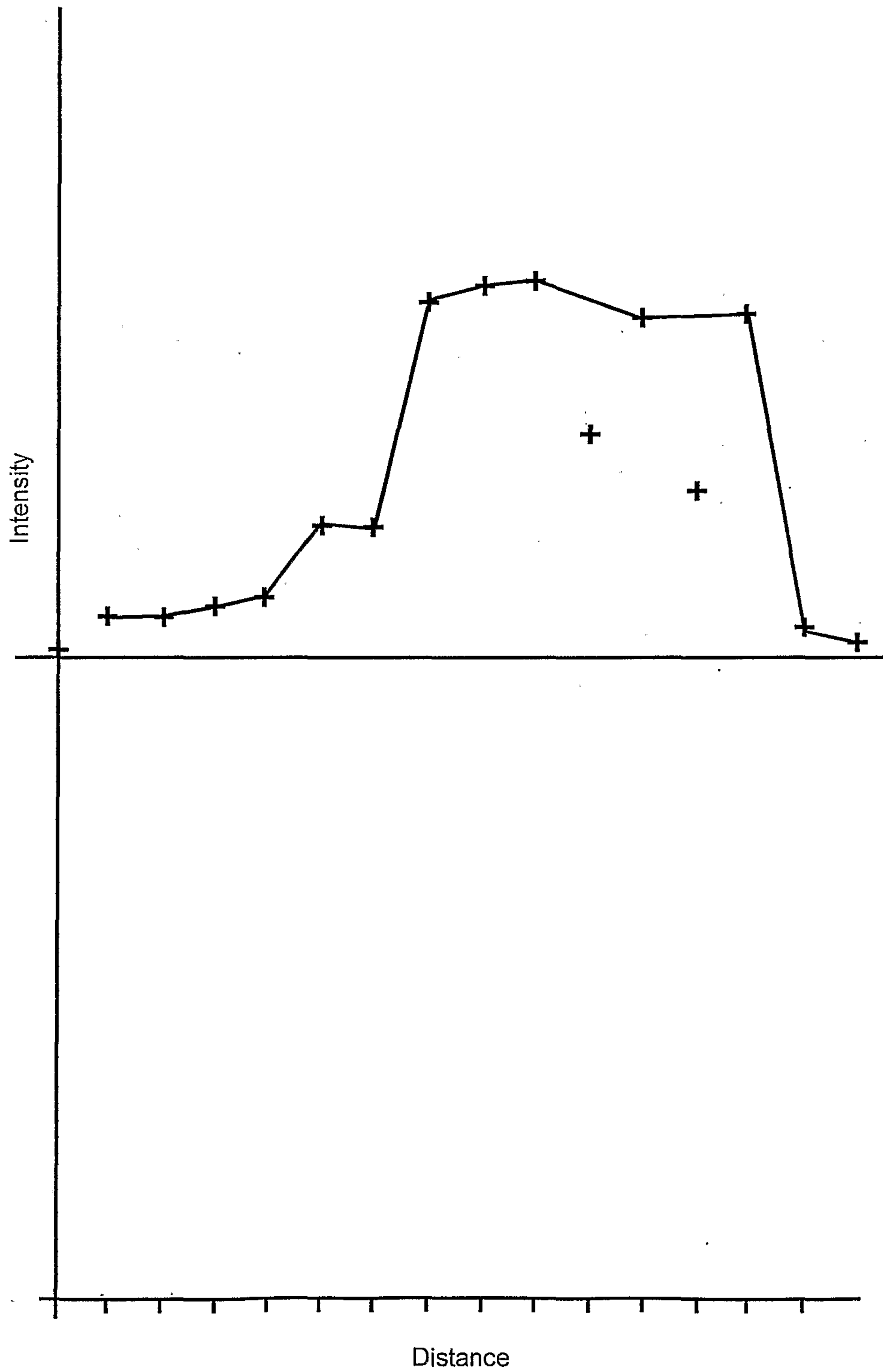
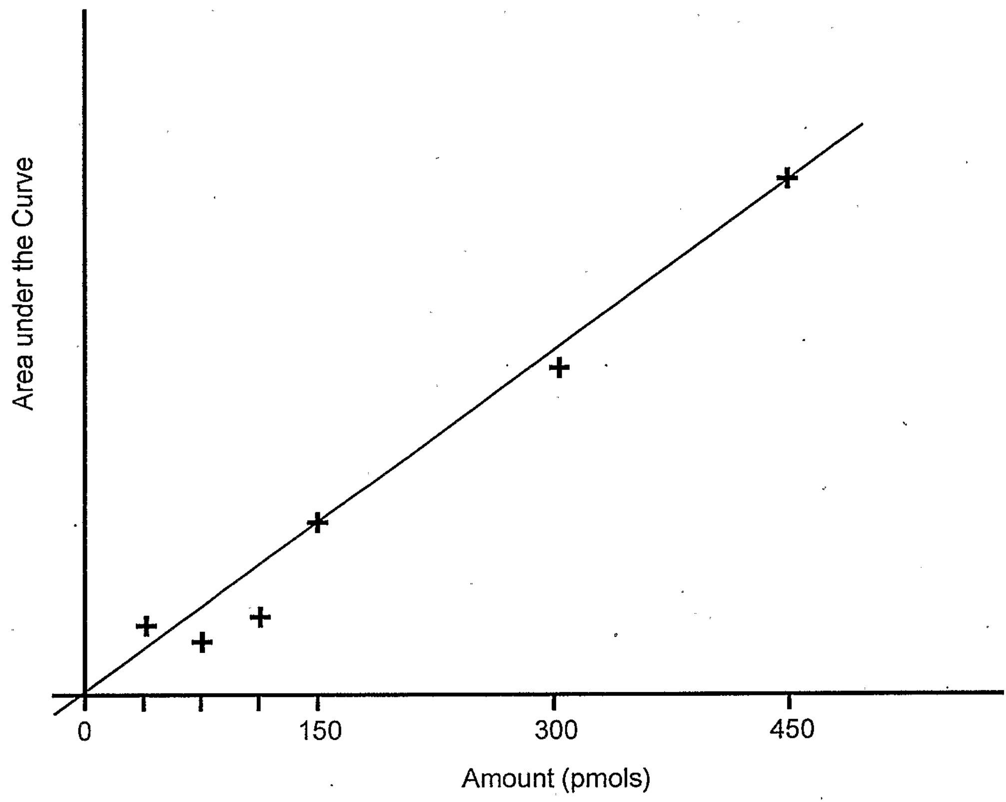
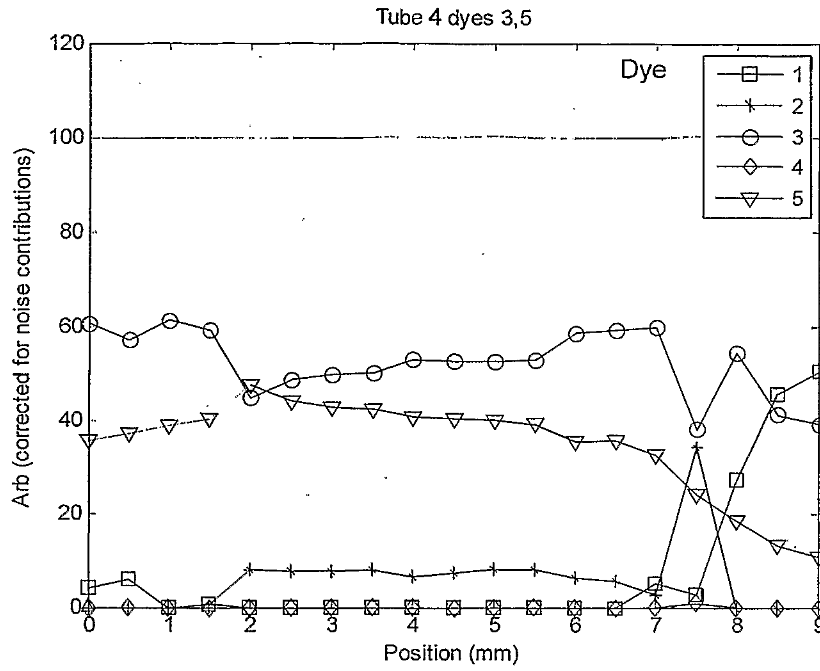


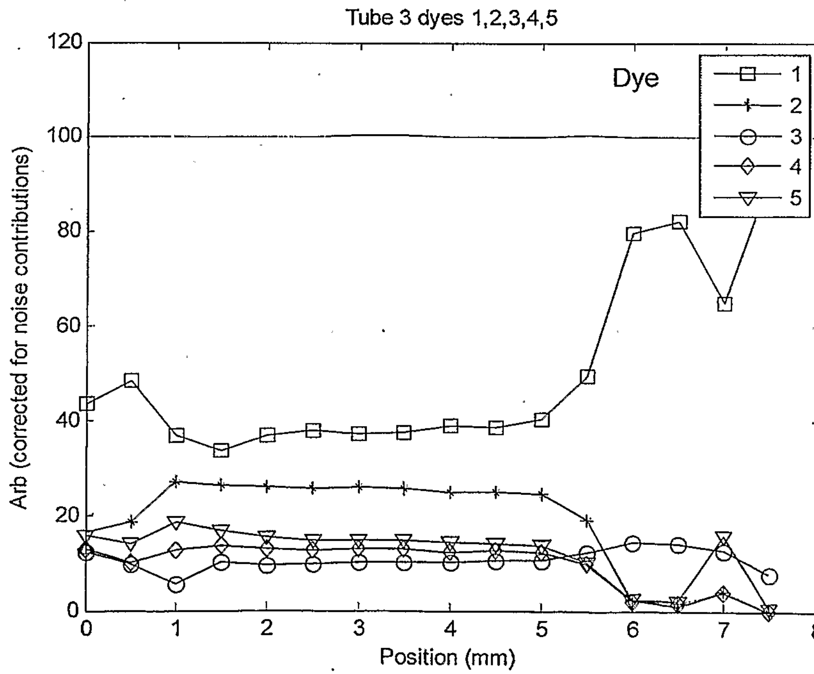

FIGURE 26




# FIGURE 27




# FIGURE 28




# FIGURE 29